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Developing Portable Tools Based on Magnetophoresis for Accessible Clinical Diagnostics

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Modern technological advancements allow us to study human cells with an extraordinary level of detail and accuracy. However, the impact of these technologies on public health remains limited by their complexity, cost, and inaccessibility. Hence, there is a need for user-friendly, inexpensive tools that can be used at the point of care, in limited-resource settings, and at home. Therefore, we aim to develop a platform technology that can perform a variety of medical diagnostics to rapidly provide important information in order to best inform medical decisions. Magnetophoresis is a phenomenon that enables precise manipulation of particles that do not necessarily have magnetic properties by suspending them in a paramagnetic medium. In previous studies, magnetophoresis has been utilized to manipulate single cells on the basis of their inherent properties, such as density, which can be indicative of cell type, disease state, etc.; however, current setups are not compatible with the point of care, limited-resource settings, or home use. Here, we develop integrated devices that levitate cells in a magnetic field, image their spatial distribution, and interpret this distribution to return quantitative results. In Aim 1, we utilize 3D printing to prototype and develop user-friendly medical diagnostic tools based on the use of magnetophoresis to manipulate single cells, including two versions: a smartphone-compatible version and a self-contained version. Aim 2 is to expand the developed platform with flow-assisted magnetophoresis and fluorescence imaging. Aim 3 is to validate the proposed tool as a platform technology by demonstrating useful clinical applications including sickle cell disease diagnosis and white blood cell cytometry. Aim 4 is to investigate the commercial potential of this concept by speaking directly with key stakeholders in the market. Herein, we demonstrate a diagnostic platform that is user-friendly and highly accessible at the point of care, in limited-resource settings, or in the user’s own home. With further clinical validation, we expect that the proposed technology will enable personalized medicine by facilitating global access to important medical diagnostics.
Developing Portable Tools Based on Magnetophoresis for Accessible Clinical Diagnostics

Stephanie Michelle Knowlton

B.S., University of Connecticut, 2015

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Submitted in Partial Fulfillment of the
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at the
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APPROVAL PAGE

Doctor of Philosophy Dissertation

Developing Portable Tools Based on Magnetophoresis for Accessible Clinical Diagnostics

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2019
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Chapter 1: Introduction

Section 1.1: Specific Aims

The current trends in medicine point toward a paradigm shift away from reactive medicine toward personalized, patient-centered medicine. This shift is facilitated by technological advancements that allow us to handle and study human cells with an extraordinary level of detail and accuracy. However, the impact of these technologies on public health remains limited by their complexity and inaccessibility. Hence, there is a need for user-friendly, inexpensive tools that can be used at the point of care, in limited-resource settings, and at home. Therefore, we aim to develop a platform technology for a variety of medical diagnostics that can rapidly provide important information to best inform medical decisions.

Magnetophoresis is a useful phenomenon that enables precise manipulation of cells on the basis of their inherent properties, such as density, which can be indicative of cell type, disease state, etc. Here, we develop integrated devices that levitate cells in a magnetic field, image their spatial distribution, and interpret this distribution to provide important medical information to the user. These features make this technology user-friendly and highly accessible at the point of care, in limited-resource settings, or in the user’s own home. Thus, we expect that the proposed technology will enable personalized medicine by facilitating access to important medical diagnostics.

Aim 1 is to develop user-friendly medical diagnostic tools based on the use of magnetophoresis to manipulate single cells. This includes two versions: a smartphone-compatible version and a self-contained version; we hypothesize that these versions of the device will be applicable to different users. The smartphone version of the device is relatively low cost because it uses the imaging and processing capabilities of the user’s own smartphone. This is particularly important for applications in developing countries and for use at home. However, in healthcare settings in developed countries, this presents issues with managing patient records and maintaining patient privacy if a doctor or practitioner has to use his or
her own smartphone. Thus, we further aim to develop a self-contained version of the device with built-in imaging and processing components that does not require any peripheral equipment.

**Aim 2** is to expand the developed platform with flow-assisted magnetophoresis by combining dynamic magnetic and microfluidic forces and implementing fluorescence imaging. We aim to expand upon the platform developed in Aim 1 by adding additional features including flow-assisted sample analysis, which can be applied to conduct dynamic sample separation in large-volume samples. We will additionally implement fluorescence imaging to provide another way to identify certain features of cells. These developments will focus on maintaining the low cost of the system, improving usability, and introducing functionality.

**Aim 3** is to validate the proposed tool as a platform technology by demonstrating three useful clinical applications. Here, we aim to develop a platform technology—a tool that can be used for a range of applications. Thus, we propose to develop three specific clinical diagnostics applications by implementing modifications to the hardware, software, and the testing reagents used. Specifically, we will demonstrate sickle cell disease (SCD) diagnosis and white blood cell cytometry. Ultimately, this will allow a single device to be applied for a variety of diagnostics in clinical and home settings.

**Aim 4** is to investigate the commercial potential of this concept by speaking directly with key players. The ultimate goal of engineering research is to positively impact the world. In our case, we aim to develop a tool to conduct clinical diagnostics on site to improve accessibility to patients. Thus, it is critical to develop these devices while considering the needs of the end users (doctors, clinicians, and patients) and others who play important roles in the clinical diagnostics market (insurance companies, regulators, and hospital practitioners). Therefore, we will conduct customer discovery according to the NSF I-Corps Framework, which is designed to accelerate the translation of basic-research projects into commercial products. This work comprises in-person interviews structured to validate critical hypotheses regarding our product design. Achieving this aim is an important step in the translation of a new concept to an impactful product.
Section 1.2: Novelty

Here, we aim to create a novel, broadly applicable technology to interrogate and monitor cells based on their inherent properties. The compact and portable diagnostic platform functions based on a technique called magnetophoresis by a sample of cells (such as a drop of blood) are collected from a patient and levitated in a magnetic field, imaged, and analyzed to return quantitative results. The sample preparation involves only sample collection (such as a finger stick for blood analysis), simple mixing with pre-mixed reagents, loading into a capillary tube via capillary action, and inserting the tube into the device; no staining, filtering, etc. are required. Algorithms running on the device quantitatively analyze the cell distribution and quantitates and compare them to “normal” measures defined based on samples from healthy patients. In this way, the device provides medical diagnostic information to the user within 10 minutes. In contrast to more complex instruments, this user-friendly, low-cost, and portable tool is compatible with the point of care (POC) and can provide rapid results. Thus, the proposed work will result in a broadly applicable and widely accessible platform for high-resolution, real-time monitoring and quantification of a cell population for clinical diagnostic applications at the POC. These efforts are enabled by 3D printing for rapid prototyping. The continuous process of computer-aided design (CAD), 3D printing, testing, and re-design facilitates agile technology development at a low cost and a high throughput. Herein, we highlight how 3D printing is leveraged in the technology development.

Section 1.3: Significance

We propose a novel platform technology for high-resolution interrogation and monitoring of cell populations or single cells. This user-accessible platform will provide extensive cytometric capabilities based on the inherent cellular characteristics. Using this technology, tests can be performed rapidly in the physician’s office so that diagnostic results can be returned while the patient is still in the office. This new process of diagnostic testing will allow medical treatment to be administered promptly and better inform a physician’s medical decisions. Further, the device is automated (thus reducing the risk of user error) and user-friendly enough to be performed by doctors or nurses with minimal training. Our vision is to create a
platform technology, leveraging a sensitive and specific yet versatile approach to enable a broad range of medical diagnostic applications within a single platform. To demonstrate the broad potential, we will demonstrate three potential clinical applications: (i) a test for SCD to diagnose and monitor patients with the disease and (ii) detection and quantification of single cells, such as WBCs in a cell population.

Herein, we mainly investigate the potential applications in the United States to provide rapid results at the POC. Moreover, as future potential application areas, Figure 1 summarizes the areas of need for better tools to improve worldwide access to accurate medical diagnostics. Specifically, there is a need for diagnostic tools that can be implemented either in the home or medical clinics, particularly those with limited resources. When used at home, this technology can enable people to have more control over their own health or particular medical conditions. It can further collect information at regular intervals to provide a physician with more information to better treat the patient while collecting information over time to provide a deeper understanding of human health. Moreover, this technology has the potential to be used in fundamental biological research to interrogate cell properties in real time. In this way, we hope to accelerate innovation in biomedical research and offer a pathway for recent and future discoveries in cellular biology to be translated into clinical practice.

Figure 1 Applications of portable diagnostics (left) In-home testing and reporting of results to the physician and (right) use in clinical settings, including low-resource clinics settings.
Reproduced, with permission, from (Yenilmez, et al., Adv Mat Tech, 2016a)
Chapter 2: Background

The current state of technology allows us to handle and study human cells with an extraordinary level of detail and accuracy. However, the impact on public health remains limited by the challenges associated with the translation of these technologies into clinically applicable tools. Currently, many medical diagnostic procedures are inefficient and inaccessible to a large population in the world. Many procedures require advanced, expensive testing equipment and labor-intensive protocols that must be performed by trained technicians. Thus, in many cases, patient samples are collected in a physician’s office and sent to centralized labs in remote locations for testing. In developing countries, this means that it may take several days for a physician to receive the results of a test, causing delays to patient treatment and, thus, complications in the course of treatment. Further, this makes many diagnostic procedures very costly and inefficient, placing a tremendous burden on the health budget of insurance payers. Moreover, this complexity makes many important diagnostics inaccessible in developing countries, where there are little funding and resources and patients often have to walk several miles to reach a clinic. Thus, the need for advanced equipment, training, and time limits the ability to obtain important information that is needed to make informed medical decisions.

However, we predict that there will soon be a paradigm shift toward personalized, patient-centered medicine. Therefore, we propose to develop accessible platform technologies for medical diagnostics based on the use of magnetophoresis for single-cell manipulation. The proposed tool is simple and inexpensive enough for use at the POC, in the home, or in low-resource settings like those in developing countries. We demonstrate several potential applications of the platform: sickle cell disease (SCD) diagnosis, white blood cell (WBC) cytometry, and cancer cell detection. Moreover, in the design of translatable technologies, it is essential to consider all social, economic, and environmental factors influencing not only the features of a proposed technology but also the path to its implementation. Thus, we additionally conduct interviews with important influencers in the market.
Section 2.1: Diagnostic applications

Sickle cell disease

One area of particular need is SCD, which is most common in sub-Saharan Africa (around 24% carry the trait, and 2 of every 100 children born have SCD)[1]. In people with SCD, red blood cells (RBCs) survive only 10–20 days (compared to 120 days in patients without the disease), resulting in a low RBC count and low hemoglobin level (anemia)[2]. SCD is caused by a mutated form of hemoglobin called HbS. Under deoxygenation, HbS undergoes polymerization, which induces a shape change in the RBCs to a rheologically unfavorable sickle form. This change can inhibit blood flow and cause organ damage, stroke, infection, and pain. Often, if SCD is left undiagnosed, children can experience silent strokes, which have no symptoms yet cause significant morbidity and mortality[3].

The RBC shape change is associated with a density change arising from a combination of HbS polymerization and a change in the activity of cation transport systems[4]. Under deoxygenated conditions, HbS molecules polymerize to form long, rigid fibers that aggregate and cause RBCs to take on the characteristic sickle shape[5]. HbS polymerization increases the permeability of the cell membrane to cations such as Na\(^+\), K\(^+\), Ca\(^{2+}\), and Mg\(^{2+}\) [6]. This change triggers KCl export by Ca\(^{2+}\)-activated K\(^+\) channels (also known as the Gardos channels), resulting in acidification and dehydration[4], [6]. In RBC subpopulations of reticulocytes and young RBCs, which express high numbers of K-Cl cotransporters (KCCs), the pathway becomes overactive in response to acidification[7]: the KCC pumps KCl out of the cell, resulting in further dehydration and a decrease in cell volume[4], [7]. Further acidification results in a positive feedback loop of KCC-induced acidification and further dehydration[7]. As a result of this process, RBCs in people with SCD have been observed to become dehydrated and increase in density[8].

According to one study[9], a subset of the RBCs undergo dehydration, creating a hyperdense cell fraction (i.e., >1.114 g/mL) comprising many irreversibly sickled cells while other RBCs dehydrate to an intermediate density (i.e., 1.090–1.114 g/mL). In another study[8], density gradient centrifugation was used to isolate fractions based on density: the four fractions isolated were found to be enriched with
reticulocytes (the least dense and slightly less dense than normal RBCs), discocytes, dense discocytes, and irreversibly sickled cells (the most dense), respectively. While other studies utilize different density thresholds to define subpopulations of RBC, it is consistently reported throughout the literature that a subset of the RBCs in people with SCD exhibit increased densities.

In the proposed diagnostic test (Figure 2), sodium metabisulfite (SMBS) is used as an oxygen scavenger to induce the deoxygenated T-state of hemoglobin[5]. As is previous studies, a concentration of 10 mM was selected. SMBS initiates these pathways that result in RBCs dehydration and an increase in RBC density. A density-based method involving centrifugation in a multiphase system has also been proposed to separate sickled cells based on their relatively high densities[10]. SCD diagnostics can also be performed using alternative techniques. For example, SCD can be detected based on a solubility test that detects the aggregation of abnormal hemoglobin[11]. In addition, the hemolysis assay is based on the lysis of SCD RBCs under deoxygenated isosmotic nonelectrolyte conditions[12]. Paper chromatography can alternatively be used to distinguish SCD patients from those carrying the trait and from patients without the disease based on a characteristic staining pattern on paper[13]. However, unlike these alternative techniques, the proposed tool provides rapid and automated analysis at a relatively low cost without a need for specialized training or user interpretation.
White blood cell cytometry

White blood cells (WBCs) are generally responsible for fighting infections. In the blood, WBC counts above or below the normal range (3500–11000 µL\(^{-1}\)) can be a useful diagnostic indicator: high WBC counts may indicate infection, an immune disorder, or leukemia while low WBC counts may indicate an autoimmune disorder or a condition that damages the bone marrow, such as leukemia. Tracking WBC levels is critical in patients undergoing chemotherapy or those with human immunodeficiency virus (HIV). Clinically, WBC cytometry may be done visually under a microscope by a trained user. Alternatively, density-based separation, or fractionation, of RBCs and WBCs can be performed, in which the denser RBC fraction is separated from the less-dense WBC fraction via centrifugation in a density gradient medium. However, this protocol requires several steps by the user and downstream cell counting to quantify the isolated WBC population. Various medical devices have been developed to perform the analysis; however, these devices generally require reagents or stains to distinguish the cell population of interest. Laboratory devices, such as the Sysmex XE 2100, use flow cytometry to quantify the WBC population. In this assay, a fluorescent polymethine dye is added to stain nucleic acids and organelles and a surfactant is used to lyse the RBC membranes[14]. Hence, WBCs can be differentiated from RBCs based on the fluorescence intensity and forward scatter (related to size) and quantified. However, this technology is very costly and requires specialized training, making it incompatible with POC settings. To address this issue, a portable, on-site device, the Hemocue WBC System, counts WBCs from whole blood loaded into a cuvette, where the RBCs are lysed and a stain is introduced to identify the WBCs. The sample is then optically imaged to count the number of stained cells. While this POC-compatible device has shown promise in making this test available on-site, the list price is over $600[15], making it inaccessible particularly to those in developing countries.

Section 2.2: Single-cell manipulation

Cell sorting and cytometry, or quantification of the number of cells of interest in a biological sample, is an essential biomedical tool used both in the laboratory and by clinicians as a medical diagnostic test. Label-
free approaches eliminate the need for cell markers and allow separation based on intrinsic cell properties. These include dielectrophoresis[16], which is used to separate cells based on their dielectric properties and size, and Raman-activated cell sorting[17], which sorts cells based on their intrinsic biochemical profiles. Microfluidic-based techniques, such as inertial focusing[18], separate cells based on lift forces, acoustic-based cell sorting[19] differentiates cells based on a combination of size, density, compressibility, and optical separation[20]. There have been attempts to precisely measure the fundamental material properties, such as the density, of single living cells. One such approach involves nanofabricated, suspended microchannel resonators; this approach suffers from low throughput and the necessity to use a sophisticated pump mechanism to transfer cells between fluids with different densities[21]. Other approaches include phase-shifting interferometry[22], digital holographic microscopy[23], quadriwave lateral shearing interferometry[24], [25], and quantitative phase tomography[26]. Despite their success in quantifying cell density, these technologies cannot readily measure subtle changes and, due to the need for peripheral devices, are not readily applicable in POC settings.

Section 2.3: Magnetophoresis

Magnetophoresis is a technique that can be used to suspend or “levitate” objects in a paramagnetic solution within a magnetic field; it is also commonly referred to as magnetic levitation. The mechanism depends on the relative density between the levitated object and the suspending medium. Thus, the levitation height of a suspended object can be used to characterize properties of either the levitating objects or the suspending medium. In one manifestation of

Figure 3 Magnetophoresis of micro-objects
Examples for objects that are denser (left) and less dense (right) than the suspending medium
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this concept, micro-objects are suspended in a paramagnetic medium and placed between two magnets oriented with like poles facing each other; here, there is a net-zero magnetic field at the centerline between the magnets and the magnetic field increases toward the magnet surfaces. Thus, a magnetic force, \( F_m \), acts on the micro-object in the direction of the centerline between the two magnets (Figure 3, orange dotted line) with a magnitude that varies as a function of the micro-object’s location in the magnetic field. The gravitational force, \( F_g' \) (also called a buoyant force), is either downward (in the case of microspheres that are denser than the suspending medium, at left) or upward (in microspheres that are less dense than the suspending medium, at right). In addition, a drag force, \( F_d \), opposes the motion of the object until the object reaches a line of equilibrium below the centerline (in the case of denser objects, at left) or above the centerline (for less-dense objects, at right). The combination of these forces moves the micro-object to an equilibrium height in the field at which \( F_m \) and \( F_g' \) are equal and opposite to each other and \( F_d \) has a zero magnitude. By this approach, micro-objects can be spatially confined in 3D magnetic traps.

This technique has been applied by the Whitesides research group to separate objects based on density and magnetic susceptibility relative to those of a suspending medium[27], [28]. These applications include separating foods, including determining the fat content in milk, cheese, and peanut butter, and comparing grains on the basis of density[29]; characterizing trace particles as forensic evidence[30], crystal polymorphs[31], and polymer composition[32]; monitoring chemical reactions over time[32]; measuring protein binding[33]–[35]; material characterization and quality assessment[36]; and performing quantitative immunoassays via the binding of antigens and antibodies and observing the density change upon binding[37].

However, these earlier magnetophoresis-based studies were performed using large setups and strong magnets, each measuring 5 by 5 by 2.5 cm, and the quantification of density was done based on the levitation height as measured with a ruler[9]–[17]. More recently, a small-scale magnetophoresis platform was developed by the Demirci group. In this setup, the magnetic field is created by 2 by 5 by 50 mm
magnets[38], which is 125 times smaller in volume than the previously used magnets. Magnetophoresis on a micron scale reduces the size of the apparatus, mitigates the risk of the setup interfering with surrounding electronics, minimizes the amounts of reagents needed (and therefore the cost of each test), reduces the equilibration time, and introduces the ability to levitate and monitor micron-sized particles. This device was shown to be compatible with single-cell manipulation to detect SCD, determine the age of red blood cells, and separate cancer cells. However, imaging with this device requires a microscope, which limits its portability. It should be noted that magnetophoresis is based on the diamagnetic properties of the levitating particles. For example, microspheres do not exhibit any magnetic properties (i.e., they are diamagnetic). Similarly, most cells are diamagnetic. However, RBCs contain hemoglobin, in which the heme group contains iron. The iron is bonded to the rest of the heme group and globin chains by covalent bonds while in the oxygenated form but, rather, by ionic bonds while in the deoxygenated form. Thus, the oxygenated form is diamagnetic due to the absence of unpaired electrons. On the other hand, deoxyhemoglobin has four to five unpaired electrons per heme group, making it slightly paramagnetic[39]. This should cause deoxygenated RBCs to levitate slightly lower in the magnetic field compared with oxygenated ones as their levitation height depends on both their density and their magnetic susceptibility. However, this is not expected to be an issue for the selected applications: in the WBC cytometry application, any RBCs that are deoxygenated would levitate lower, away from the WBC band. Further, in the SCD application, all RBCs are deoxygenated, so the differences observed would be due only to the SCD status, implying that SCD and normal RBCs could still be distinguished.

Section 2.4: 3D printing for rapid prototyping

3D printing enables a method of agile design and fabrication of custom devices and tools. Parts can be fabricated at a high-throughput and a low-cost. In many cases, it serves as a promising alternative to traditional fabrication techniques such as tooling and machining for large-scale parts and lithography for micro-scale parts. The continuous process of computer-aided design, 3D printing, testing, and re-design
enables efficient design iterations in the technology development stage. In this way, 3D printing is expected to promote rapid innovation in diverse fields of basic science and engineering.

There are several styles of 3D printing; the main types are extrusion, inkjet, and stereolithography. Extrusion printers extrude a continuous stream of material, often a melted thermoplastic or a hydrogel, in a desired pattern on a stage by moving a printing head along the X,Y-plane and the material solidifies upon cooling or a chemical reaction, respectively. This is repeated in a layer-by-layer manner to build up the desired structure in 3D. Inkjet printers similarly deposit material in a layer-by-layer manner but instead create droplets of the material through one or more printing heads as they move across the stage. Finally, stereolithography printers are based on the application of light in a specified 2D pattern to a pool of photocrosslinkable liquid resin to cure the resin onto a stage, which is moved vertically to build layers.
Chapter 3: Methods

Section 3.1: Smartphone-compatible setup

The smartphone density analysis platform[40] (Figure 4) consisted of a 3D-printed smartphone attachment custom designed to attach to a Samsung Galaxy 4 (or another smartphone with a simple re-design). It was printed on a Form 1+ high-resolution stereolithography 3D printer with photoreactive resin (Formlabs, Somerville, MA) (Figure 4a-c). The platform included a magnetic field into which a sample is loaded, an optical lens that magnifies an illuminated image of the sample onto the built-in camera in a

![Figure 4](image)

**Figure 4** The smartphone-attachable platform to separate micro-objects based on their densities (a-b) Front and back views of the 3D printed magnetic levitation platform. (c) Imaging of levitating microspheres using the smartphone camera and the 3D printed attachment. (d) 3D schematic diagram of the smartphone attachment, where a disposable microcapillary filled with the sample is inserted from the side for imaging. (e) Magnified illustration of the platform showing the LED illumination, a ground glass diffuser, two rare-earth permanent magnets with the same poles facing each other, and 3D printed lens holder. Microspheres in the magnetic field are levitated and confined and those outside of the magnetic field are distributed randomly. (f-g) Captured image of 10 μm polystyrene microspheres (scale bar is 100 μm), (f) at t = 0 and (g) levitated and confined at equilibrium.

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smartphone, and a custom-developed Android application to analyze the image and quantify the levitation height of the sample. The smartphone camera was aligned to capture a magnified image of the levitating micro-objects through an additional lens positioned between the sample and the camera lens cover (Figure 4d-e). In the presence of the magnetic field generated by two permanent magnets, the randomly distributed microparticles inside the microcapillary move toward an equilibrium line and form a horizontally aligned cluster (Figure 4f-g).

### Optical configuration

The optical component of the attachment includes a battery-driven LED to illuminate the sample. The LED is connected in series to a CR 2032 3V battery (CR2032, Panasonic, Newark, NJ) and a slide switch (GF-1123-0025, CW Industries, Southampton, PA) for user operation. From the LED, the light travels through a 1500 grit ground glass diffuser (DG05-1500, Thor Labs, Newton, NJ), selected to provide uniform illumination to the sample across the entire field of view (FOV). A 4-f imaging system is implemented to magnify the image of the sample and project it onto the built-in smartphone camera (Sony IMX135, 1.12μm pixel pitch, 4208x3120 pixels). The image is magnified by an external aspheric lens (87–161, Edmund Optics, Barrington, NJ) with an outer diameter of 6.33 mm, effective focal length of 4.03 mm, and numerical aperture of 0.64. This lens is fixed into the 3D printed attached and aligned with the built-in camera lens (which has an effective focal length of 4.2 mm) such that the focusing distance between the two lenses and between the lens and the sample is optimized experimentally and fixed in the 3D printed attachment design. Fine focusing is effectively achieved by the autofocus capabilities of the built-in camera application on the Samsung Galaxy S4. The FOV captures the entire height of the magnetic field at a 2X digital magnification, including the 0.7 mm range of levitation for the sample in the microcapillary tube and can image microspheres when they are distributed homogeneously upon entering the magnetic field and as they approach equilibrium. A custom-developed Android application then analyzed these images to determine the levitation height and estimate the density.
This platform is supported by a custom-developed Android application running on the same smartphone. The Android application was developed in Android Studio, which is an Android Developer Tool (The Android Open Source Project, developer.android.com). The custom-developed application processes the array of pixel intensities, where the x-coordinate corresponds to the horizontal position and the y-coordinate corresponds to the vertical height. The algorithm performs two main steps:

1. Scanning the pixels in each row, averaging the intensities of pixels, and storing them in an array indexed by the y-direction height of the row in pixels.

2. Scanning the pixels in each row, finding the gradients of the pixel the intensity changes in the x-direction, and saving them in an array indexed by the y-direction height of the row in pixels.

The first array values generally peak at three locations: (region 1) the region starting from the upper edge of the microcapillary to the top edge of the image, (region 2) the region starting from the lower edge of the microcapillary to the bottom edge of the image, and (region 3) the confinement region where microspheres are located. The second array values mainly peak at again three locations: (region 1) the top and (region 2) the bottom edges of the capillary where they meet with the magnets and (region 3) as in the first array, the confinement region where microspheres are equilibrated. Based on these two arrays, region 3 is identified. A Gaussian distribution is fitted to the pixel intensity data and its mean and standard deviation (σ) are calculated in pixels. Based on the standard deviation, the width of the bead confinement (~4σ) is estimated.

The magnet boundaries are located by iteratively comparing the gradients of pixel intensities in the y-direction of one pixel with that of the neighboring five pixels. The magnet boundary towards the top of the image (henceforth referred to as the ‘top magnet’) serves as the reference point for calculating the levitation height of the particles of interest. The same technique is used to identify the edges of the microcapillary as is used to identify the magnet bounds. The gradient of pixel intensity of the first pixel
near the levitating objects is successively compared with the neighboring pixels towards the top until the
top magnet is encountered or the gradient difference exceeds 20% to locate the upper edge of the
microcapillary. Similarly, the lower edge is located. The distance between the inner edges of the
microcapillary from top to bottom is known to be 0.7 mm. Based on this constant value and the number of
pixels between the edges of the microcapillary, a conversion factor for pixels to mm is obtained. This is
then used to convert the levitation height and standard deviation from pixels to mm and these values are
displayed in the user interface in millimeters.

**Microsphere levitation**

For validation, 10 μm Polybead polystyrene blue dyed microspheres (Polysciences, Inc., Warrington, PA
18976) were used to test the time-dependent magnetic levitation and the effect of the gadolinium
concentration on levitation height. To correlate density to levitation height, eight density standard
microspheres were obtained: 0.96, 0.98, 0.995, 1.13, 1.025, 1.050, 1.070, and 1.090 g/cc (Cospheric,
Santa Barbara, CA 93111). To perform the magnetic levitation tests described here, microspheres are
suspended in a Gadolinium-based (Gd) paramagnetic medium, Gadavist (Bayer, Whippany NJ 07981)
diluted to the desired concentration in Hank’s Balanced Salt Solution (55021C, Sigma, St. Louis, MI).
The paramagnetic solution used for all experiments presented here, Gadavist, is currently employed for
MRI investigations in humans throughout the world. The gadolinium medium is non-toxic, iso-osmolar at
the concentration used for imaging, and fully compatible with human blood cells[38]. The sample is then
drawn into a 0.98 mm by 0.98 mm square glass disposable microcapillary with 0.14 mm walls (8270–50,
Vitrocom, Mountain Glass, NJ) by the capillary effect. The ends of the capillary tube are sealed with
Critoseal to prevent leakage (215003, Leica Biosystems St. Louis, LLC, St. Louis, MO). Then, the sample
is slid into the 1 mm space between magnets.

To quantify the microsphere focusing over time, the upper and lower limits of the region in which the
microspheres were confined at periodic time points was measured. Nine different concentrations of
gadolinium paramagnetic solution were tested between 20 mM gadolinium in (a) to 250 mM in (i).
Microspheres were added at 2.5% to each solution and levitated as described previously. Images were taken every 15 seconds with time 0 corresponding to insertion of the sample into the magnetic field until the microspheres appear to reach equilibrium (no significant vertical movement of microspheres). Each data point is an average over six trials. The difference between the top and bottom limits at each time point, or the width of confinement, was calculated. An exponential decay regression was fit to the confinement width data to quantify the time dependence of microsphere levitation and confinement. All regression equations had R-squared values greater than 0.94. This regression was used to determine the equilibrium time as the time required for the confinement width to reach 1.5 times the height of a single bead (10 μm), or 0.015 mm. The height at equilibrium was then calculated as the average between the upper and lower limits of confinement at the time point immediately following the equilibrium time.

To quantify the effect of particle size on equilibration time, three different sizes of polystyrene microspheres were levitated: 5.35, 10.4, and 20.0 μm in diameter. Microspheres were suspended in 50 mM gadolinium solution and levitated in the apparatus as described previously. Images were taken starting when the microcapillary was inserted into the magnetic field (t = 0) and periodically until the microspheres reached equilibrium and the equilibration time was calculated as described previously.

To correlate micro-object density to levitation height, the eight density standard microspheres ranging from 0.96 to 1.090 g/cc were levitated as described previously. The equilibrium levitation height was quantified as the average levitation height of 60 microspheres taken over six trials relative to the bottom magnet. A linear regression line was used to calibrate the experimental equilibrium levitation height to the known microsphere density. Calibration curves were determined in five concentrations of gadolinium solution: 12.5, 25, 50, 100, and 200 mM gadolinium.

**Evaluating the optical performance**

To characterize the optical performance, an image of the levitating micro-objects is magnified by the secondary lens and captured using the built-in camera of the smartphone. The useful FOV (and the area in
which the application will analyze the levitation height) has been characterized in terms of the microsphere sharpness, image distortion, and uniformity of illumination as a function of horizontal position within the acquired FOV. Vertical image distortion was determined by measuring the distance between the magnets in 10-pixel-wide vertical cross-sections iteratively across the FOV. The percent increase in the FOV height was calculated for each horizontal cross-section as the percent difference between the measurement taken in the cross-section and the same measurement taken at the centerline. Uniformity was calculated by taking the average of the pixel intensities in 10-pixel-wide vertical cross-sections of the area between the magnets iteratively across the FOV. Sharpness was determined as the maximum change in intensity between 2 adjacent pixels on the edge of a microsphere along a line drawn radially from the center of the microsphere outward. The sharpness of each microsphere was plotted as the steepest pixel gradient versus the horizontal distance from the center of the steepest gradient.

### Measuring the temperature distribution

Further, the temperature distribution within the setup was mapped to ensure that the temperature (which affects the density of the sample and the paramagnetic medium and, therefore, may influence the results) does not vary significantly over the operating period. To evaluate the potential effects of thermal flux from the smartphone due to running the smartphone application and LED, several representative locations were identified and marked at the back surface of the smartphone. The temperatures at these locations were measured using a thermocouple temperature meter (B&K Precision) as a function of time while the LED was on, the screen was on, and the application was running. Based on these results, the camera was identified as the most critical component in terms of heat accumulation. The cooling times required between separate trials were therefore determined based on the camera’s cooling time. The effect of thermal release from the camera on the sample in the microcapillary was quantified with a room temperature 50 mM gadolinium solution. At time 0, the application was opened, the LED was turned on, and the capillary tube was placed between the magnets. The capillary tube was kept inside the holder for different time periods: 2, 5, 10, 20, 30, 40, 50, 60, and 120 minutes and after each elapsed time, the tube
was removed and the temperature of the capillary tube and the gadolinium solution inside the tube were measured immediately. This experiment was repeated six times for each elapsed period with an optimized time to cool to the originally recorded temperature (25.6 ± 1°C) between experiments.

### Section 3.2: Self-contained setup

The self-contained platform[41] contains three main components: a magnetophoresis module, optical components, and a processing unit (Figure 5). Like in the smartphone-based version described above, the magnetic levitation component consists of two magnets fixed in a laser-cut holder and separated 1.1 mm apart such that the like poles are facing each other and the magnetic field is oriented vertically (parallel to the gravitational force). As in the smartphone-based setup, the sample is suspended in a paramagnetic medium and loaded into a square glass microcapillary tube via capillary action, then inserted between the magnets. The magnetic field causes the sample to levitate according to the density of the particles, where denser particles levitate lower than less dense particles. Images of the levitating particles are captured using a custom optical system containing a variable-intensity light-emitting diode (LED), light diffuser, optical lens, and camera unit. The images are then processed using the onboard Raspberry Pi, which is a compact, low cost, single-board computer. This embedded computer runs on a Linux operating system and is loaded with a custom-developed Python code using both OpenCV and custom libraries for image processing and analysis. The embedded system functions to detect the levitation location of the particles in the FOV and use this value to estimate the densities of the particles. The magnetic levitation device casing was custom designed using CAD software and 3D printed using an Objet30 Prime (Stratasys, Eden Prairie, MN), a high-resolution PolyJet-based 3D printer with a 32 μm layer height.

### System configuration

Within the casing, a Raspberry Pi embedded computer and TFT display (Adafruit, New York, NY) are stacked and fixed in place. A 5-Megapixel camera module (v1, 1367, Adafruit, New York, NY) is also placed in the device and connected to the board via a ribbon cable. The camera module is modified by
taking apart the lens and adding a custom extension tube between the sensor and the lens. This adjustment increased the magnification of the image on the sensor considerably, thus making it unnecessary to use an additional lens which would decrease the image quality and increase the cost. The magnetic levitation module is assembled from two 2 mm by 5 mm by 50 mm NdFeB permanent magnets (K&J Magnetics, Inc., Pipersville, PA), which are magnetized through the 5 mm thickness. The magnets are enclosed in a heat-shrink tubing and fixed 1.1 mm apart by two laser-cut acrylic supports with their magnetic axes aligned with gravity and their like poles facing each other. This magnet module is inserted into alignment

**Figure 5 Schematic of self-contained magnetic levitation platform**

(a) Top view of the interior design of self-contained device including the magnetic levitation module, optical components (LED, diffuser, lens, and camera), and the processing unit with screen. (b) Close-up view of the magnetic levitation imaging platform. The sample is illuminated by a LED through a diffuser. An image of the sample passes through a lens to the camera, where the image is captured and transferred to the processing unit. (c) 3D-printed prototype of the compact and self-contained magnetic levitation and analysis platform. The user inserts a sample of microparticles suspended in paramagnetic solution in a glass microcapillary and controls the device using four buttons on the top face as well as a LED screen displaying the user interface. (d) Placement of the magnets and capillary tube for testing. Two permanent NdFeB magnets are fixed in a laser-cut holder 1.1 mm apart with like poles facing each other. The sample, contained in a 1 mm square glass microcapillary tube, is inserted between the magnets to achieve density-dependent magnetic levitation of the microparticles. The sample is imaged in a ≈1 mm wide region of the capillary tube, delineated in blue.

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slots in the casing at a fixed distance from the camera module. Between the magnets and the camera, an aspheric lens (Adafruit, New York, NY) in a 3D-printed adjustable tube is placed such that the sample image is focused onto the camera. The lens extension tube is designed such that it screws into place and can be adjusted slightly via a user-accessible knob by turning the holder in the threads of the casing to achieve fine focusing capabilities when in use. Finally, a high-brightness, intensity-controlled white light LED (Adafruit, New York, NY) on a custom-printed circuit board (SeeedStudio, Shenzhen, China) are placed on the side of the magnets opposite the lens and camera in order to illuminate the sample with user-defined light intensity. A light diffuser is also placed between the LED and the magnets in order to diffuse and collimate the light. A covering is fixed over the components, allowing manual access to the charging and USB ports, button presses, and a clear view of the TFT display screen, resulting in a compact, self-contained, and user-friendly device.

**On-board image processing**

The pixel intensities are used to detect both the particles whose densities will be measured and the reference magnet and capillary edges. Pixel intensities are scanned in both Z- and Y-directions and the 1st order derivatives are calculated (where the Z-axis is parallel to the magnets and the Y-axis is parallel to the gravitational force). The average of the pixel intensities within Y-axis (vertical) slices over a Z-axis (horizontal) profile is calculated to obtain Y-derivatives. Likewise, Z-axis derivatives are obtained along Z-axis (horizontal) slices over a Y-axis (vertical) profile. Since the magnet and capillary edges lie along the Z-direction, they are filtered out in the Z-axis derivatives, which make it easier to detect the particles alone. Thus, the Z-derivative is used to calculate the average gradient at each height across a Y-profile and these means are plotted and fitted to a Gaussian curve to represent the distribution of the suspended particles. The software is capable of fitting multiple Gaussian curves to detect different particles, which can be triggered by the user.

The algorithm then outputs the mean and variance values of the fitted curve for each image, describing the average levitation height of the particles and their degree of confinement, respectively. The inner
edges of the magnets and the capillary are also automatically detected using the Y-derivative. The distance between the inner capillary edges, which is 0.7 mm, is used as a scale and the magnet edges are used for reference. The mean values are converted to millimeters using the obtained scale relative to the upper edge of the bottom magnet. The variance values are also converted to millimeters.

These variance values from each respective image are fit to a time-lapse exponential decay curve. The time point on the curve at which the variance value is 20 μm is taken as the time to equilibrium; the value for mean measured from the corresponding time frame is taken as the equilibrium levitation height. These values are displayed in the graphical user interface.

The thin-film transistor (TFT) display screen and four buttons serve as a user

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**Figure 6** User interface and data output

(a) The user interface allows the user to view the levitating particles (magnets are seen on the left and right, where the magnet on the left side of the image is the bottom magnet). From the main menu, the user can also zoom in on the image to focus the setup, capture a single image, capture time lapse images at 10 s intervals, or power the device on/off using the buttons located below the screen. (b) Captured image at equilibrium of a mixture of two microspheres with different densities. The processing algorithm is able to determine the levitation height relative to the bottom magnet and the standard deviation of the particles for one or more populations of microspheres. All measurements are scaled from pixels to micrometers using the ratio between the known inner diameter of the microcapillary, 0.7 μm, and the measured distance. (c) Demonstration of the time lapse imaging and analysis feature. Images are captured every 10 s over a number of minutes selected by the user (here, images were taken over 3 min). (d) Ports and features for user interface, including a capillary port for inserting the sample, a focus adjustment lever for fine focus of the sample, and charging and data ports.

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interface to control image capturing from the main screen (Figure 6a). When the user inputs a sample into the device, fine focus is achieved by pressing button 1 (far left button) to zoom in (5× digital magnification) and moving the fine focusing lever to find the optimal focus setting. Button 2 captures a single image of the sample and processes it to determine the levitation height and standard deviation (Figure 6b). Button 3 begins capturing an image every 10 s for a total time set by the user and processes the sequence of images (Figure 6c). By default, six images are captured; the first two buttons can be used to increment or decrement this value by an interval of six images, respectively. The images are also stored onto a USB flash drive for later use (Figure 6d). The ensuing image processing, as described in the following section, then extracts information about the sample by analyzing the series of captured images, outputting relevant information pertaining to the densities of the particles in the sample. The far right button functions as a power button.

**Characterization of the setup**

For characterization, the experiments with density-controlled microspheres conducted with the smartphone setup will be repeated to correlate the levitation height (as imaged by the built-in camera) with the density of the levitating particle. Further, the magnetic field strength and imaging capabilities of the device will be evaluated to conduct a comparison with the smartphone-based version.

**Section 3.3: Flow-assisted magnetophoresis device construction**

To enable a continuous flow of sample through the magnetic field as well as on-board imaging and analysis, the smartphone-compatible magnetic levitation device was modified to continuously flow of the sample while acquiring video of levitating cells as they pass through the FOV and conducting automated analysis in an Android application[42]. The modified setup is shown in Figure 7. Because a larger sample volume may be used than in the static levitation device presented previously, which is limited to only 0.5 µL per FOV), this version allows high-throughput analysis of large samples to detect cells with low frequency in the biological samples.
The smartphone apparatus contains a wireless-enabled microcontroller (RFD22102, RFduino, Hermosa Beach, CA, USA) that communicates with the smartphone through a Bluetooth wireless module. The microcontroller receives the set points for the micro-pump flow rate (in terms of input amplitude and frequency of the micro-pump) and LED intensity from the smartphone user interface. Then, it generates the appropriate outputs for the micro-pump controller and LED in order to achieve the set points. Two serially-connected AAA rechargeable batteries work as a 3 V DC power supply for the apparatus. The micro-pump driver receives the amplitude (as a PWM signal equivalent to 0–3 volt DC) and frequency (as a 0–100 Hz square signal) set points from the microcontroller and generates the micro-pump driving signal, which ranges between 0–250 volts in amplitude and 0–100 Hz in frequency. A new version of the Android application was developed that plays the roles of a user interface and an image processing tool.

A Bluetooth low energy (BLE) service was implemented and handled on the smartphone side while the RFduino carried out the commands sent from the application. BLE provides efficient communication between two devices while requiring less power and maintaining a high rate of data transfer. BLE services run at a frequency of 2.4 GHz with 40 channels having equal spacing of 2 MHz. Currently, all of the BLE profiles are based on GATT (generic attribute profile), providing an organized hierarchy of data with different tags, which could belong to two separate devices. This system defines the simplest piece of data as a characteristic which is then bundled into larger structures called services. A BLE connection typically has a client and one or more servers, with a client initiating any GATT requests, while the server responds. In this case, the GATT server was the RFduino and the client was the smartphone. The connection was implemented as a service on the smartphone to allow for a continuous connection over the application's lifecycle. The data collected from the user interface was bundled and sent across the BLE service with a universally unique identifier (UUID) tied to the data. On the server-side (RFduino), this data was received and unbundled and the information was sent to the respective pins on the board.
The flow-assisted magnetic separation was then optimized in terms of the flow rate to control the region of confinement of a particle population with a constant density. Then, the separation of particles by density from a mixture of microspheres with volumetric mass densities of either 0.97 or 1.12 g/cm³ was demonstrated as a proof of concept.

**Figure 7** Smartphone-attachable, flow-assisted magnetic focusing device
(a–c) Front (a), side (b), and back (c) views of smartphone-compatible flow-integrated magnetic focusing setup. (d) The components of the device: (1) magnetic focusing module, including permanent magnets, square microcapillary, lens, LED and light diffuser, (2) cellphone case, (3) electronics, including the microcontroller, pump driver, and Bluetooth receiver, (4) micro-pump holder, (5) adjustable orifice, (6) waste tube holder, (7) AAA battery holder, (8) sample tube holder, (9) dual-purpose stand and cover. (e) Schematic of the flow-assisted magnetic focusing device. (f) Cross-section of magnetic focusing module. Reproduced from (Amin et al., RSC Adv, 2016) with permission from The Royal Society of Chemistry
Section 3.4: Fluorescence-aided magnetophoresis device construction

The fluorescence microscopy-enabled magnetic levitation device[43] comprised a phone case, two LEDs, emission filters, and a shade to eliminate ambient light. The customized phone case attached the optics module and shade to the smartphone to reduce the ambient light. The optical setup for brightfield imaging was similar to that described in the smartphone-based setup. However, in the optical setup for fluorescence imaging, the sample was excited using LEDs with different wavelengths, which were placed a darkfield configuration to minimize the amount of background light captured by the camera. The light emitted from the sample travels through an external aspheric lens and an inexpensive plastic light filter and the magnified image is focused onto the smartphone camera.

The base device was the smartphone-compatible setup described above. Furthermore, the optical component, emission filter holders, and shade were printed using a Formlabs Form 1+ stereolithography (SLA) 3D printer at a resolution of 0.1 mm with black resin (Formlabs Inc., Somerville, MA, USA) to keep ambient light from influencing the imaging capabilities. Each part of the optical component contains tracks that permit easy assembly and disassembly of the device as well as cost-effective modification. To allow for both brightfield and darkfield imaging, the device's shade is easily attached and detached from the setup using tracks that align with the smartphone case. The optical component includes an electrical piece and a magnetic focusing piece. The electrical piece is designed to fit with a battery and switch and to exchange the microscope's emission filters (Figure 8a). The magnetic focusing component includes spaces for the two and borosilicate glass microcapillary tube (Square Boro Tubing #8270-050, VitroCom, Mountain Glass, NJ, USA) containing the sample (Figure 8a); for easily interchangeable LEDs to image the sample in the brightfield and darkfield configurations (Figure 8b and c, respectively); and for an aspheric lens with a diameter of 6.33 mm and numerical aperture of 0.64 mm (87–161, Edmund Optics, Barrington, NJ, USA) that magnifies the fluorescent image and focuses it on the smartphone camera. The camera application Camera FV-5 is used to image the sample.
In the optical setup for fluorescence imaging, the sample is excited using differently colored LEDs (Super Bright LEDs Inc., St. Louis, MO, USA). The LEDs are powered by two 3 V batteries (CR2032, Panasonic, Newark, NJ, USA) wired in series with a 220 Ω resistor, which provides sufficiently regulated power. To counter the high power drain of the LED, a slide switch (GF-1123-0025, CW Industries, Southampton, PA, USA) is attached between the battery and the diodes to prevent unnecessary current flow. Light is produced by the LEDs and directed towards the sample from the side to minimize the amount of background excitation light that is eventually captured by the camera.
To optimize the novel fluorescence microscope for biological applications, cells were stained with calcein (C3100MP, Thermo Fisher Scientific, Waltham, MA) and QTracker 625 (A10198, Thermo Fisher Scientific, Waltham, MA). Both of these substances stain the cytoplasm of cells and therefore were ideal candidates to test biological applications of the setup. Calcein, a green cytoplasmic stain, was used to optimize the setup for green fluorescence imaging. Breast cancer cells (MDA-MB-231, ATCC, Manassas, VA, USA) were cultured in RPMI medium, trypsinized, stained with calcein, and allowed to incubate for 30 minutes. Cells were then viewed under a laboratory fluorescence microscope to confirm incorporation of the fluorescent stain. The cells were then mixed in a 1 : 1 ratio with the 100 mM Gadavist solution in Hank's balanced salt solution for a final 50 mM Gadavist solution with the stained cancer cells. Based on the excitation and emission curves for calcein, the blue LED was used to excite the sample. The blue LED was chosen because it contained wavelengths required to excite the sample but did not contain wavelengths that may be mistaken for the green fluorescent light, unlike other possible LEDs such as an aqua or white light. To maintain the brightness of the excitation light and because the light did not contain a wide range of wavelengths, an excitation filter was not used. Three distinct, but similar, green emission filters were tested to determine the effect of the emission filter on a biological sample's image. The three different filters used were gaslight green (Roscolux #388: gaslight green, Rosco Laboratories, Sun Valley, CA), moss green (Roscolux #89: moss green, Rosco Laboratories, Sun Valley, CA), and pea green (Roscolux #86: pea green, Rosco Laboratories, Sun Valley, CA). All of these emission filters transmitted the most light around the 525 nm range emitted by calcein but showed lower transmittance for blue and red light. These samples were imaged in the darkfield imaging setup three times per test condition to analyze at least 30 cells for each emission filter, in each of the three digital channels. Images were analyzed using ImageJ by separating each picture into its constituent red, green, and blue channels and determining the signal strength for each combination of emission filter and channel. Because signal strength measures the contrast between signal and background for each test condition, this measurement indicates image quality, with high values corresponding to high quality. Additionally, the use of ImageJ for counting cells in each image digitally was investigated due to its relevance to future biological
applications for this fluorescence microscope. Because of the high pixel intensity of the cells relative to
the background, the “find maxima” function was tested at various noise tolerance levels.

A similar procedure was used to optimize the setup using red emission filters for QTracker 625, a red
cytoplasmic stain. Prostate cancer cells (PC3, ATCC, Manassas, VA) were cultured in RPMI medium
with QTracker 625 overnight. Based on the excitation and emission curves for QTracker 625, the blue
LED was again used to excite the sample. A UV LED was also tested but did not provide as clear images
as the blue LED. To maintain the brightness of the excitation light and because the blue light did not
contain a wide range of wavelengths, an excitation filter was not used. Three distinct, but similar, red
emission filters were tested to determine the effect of the emission filter on a biological sample's image.
The three different filters used were golden amber (Roscolux #21: golden amber, Rosco Laboratories, Sun
Valley, CA), light red (Roscolux #26: light red, Rosco Laboratories, Sun Valley, CA), and fire (Roscolux
#19: fire, Rosco Laboratories, Sun Valley, CA). All of these emission filters transmitted the most light
around the 625 nm light emitted by QTracker 625 but showed lower transmittance for blue and green
light. As in the calcein optimization, 30 cells were analyzed for each combination of emission filter and
digital channel using ImageJ. Signal strength and cell counting potential were again used to determine
image quality and usefulness in biological applications.

To evaluate effectiveness in a biological application, the device's ability to count cells was evaluated.
Breast cancer cells (MDA-MB-231) were again stained with calcein and allowed to incubate for at least
30 minutes. Cells in the stock solution of stained breast cancer cells were counted using a hemacytometer
to determine the cell concentration. From this stock solution, five different dilutions were created
consisting of 1%, 2%, 3%, 4%, and 5% of the stock solution with 99%, 98%, 97%, 96%, or 95% 100 mM
Gadavist solution by volume, respectively. These five dilutions were tested in the fluorescence
microscope presented here. The concentration of cells in each of the five samples was measured by
visually counting the cells in a photo taken and using the “find maxima” function in the photo's green
channel in ImageJ. The volume of the capillary was used to convert these cell counts to cell
concentrations. Concentrations measured visually and by ImageJ were compared to hemacytometer count to determine the accuracy of the device.

The limits of cell counting and cell detection were then investigated using ovarian cancer cells (HeyA8, ATCC, Manassas, VA) cultured using DMEM and stained with calcein. Again, the fluorescently stained cells were excited using a blue LED (RL3-B2030, Super Bright LEDs Inc., St. Louis, MO). The lower limit of cell counting was determined by capturing an image of a single cell in the FOV of the camera. The range of sizes of cells imaged with the smartphone camera was also assessed by staining cultured lung cancer cells (A549, ATCC) and measuring their diameters in ImageJ software. Measurements were taken in pixels and converted to μm using the 700 μm capillary interior for scale.

Lastly, the differences in two cell populations were analyzed in three ways. In the first test, a small population of QTracker 625-stained HeyA8 cells was mixed with a much larger population of calcein-stained HeyA8 cells. This setup mimics the detection of rare cells in a greater cell population. The paramagnetic agent, Gd, was added to the mixture to give a final concentration of 100 mM Gd. The sample was illuminated by a blue LED (RL3-B2030, Super Bright LEDs Inc., St. Louis, MO) and imaged using no emission filter, the gaslight green emission filter, and the fire emission filter. Images were also further filtered, digitally, in ImageJ to separate the red and green channels for each stain. The second test conducted between two cell populations mimics circulating tumor cells in the bloodstream. Cultured HeyA8 cells were again stained with calcein and mixed into diluted blood obtained via finger prick. Blood samples were obtained from humans with informed written consent; all experiments were performed in compliance with the relevant laws and institutional guidelines and all procedures were approved by the University of Connecticut Institutional Review Board Protocol #H15-048. The cells, which separate vertically in the magnetic field based on their different densities, are imaged in both brightfield and darkfield conditions. The third, and final, condition is designed for the use of an acridine orange stain for cell identification. Acridine orange stains both double-stranded DNA fluorescence green and single-stranded RNA fluorescence red[44]. Due to their high replication rate, cancer cells tend to be
stained more brightly red than other cells[44]. Given this trend, A549 and non-cancerous 3T3 cells (ATCC, Manassas, VA) were stained with acridine orange. Acridine orange was diluted to a concentration of 0.1 mg mL\(^{-1}\) in phosphate buffered saline, and stained cells in suspension were incubated at 37 °C for at least four hours. After washing twice and resuspending in Hank’s balanced salt solution each solution containing one of the three types was made paramagnetic by the addition of 1 M Gd to reach a final concentration of 100 mM Gd. Each cell type was imaged when excited by a blue LED with no emission filter in order to capture all emission wavelengths. Images were split into red, green, and blue channels in ImageJ, and the signal strength in the red and green channels was measured for both cell types in order to determine the red-to-green ratio in both acridine orange-stained populations.

**Section 3.5: Sickle cell disease testing**

**Sample collection and testing**

Whole blood was obtained from subjects previously diagnosed with SCD as well as presumably healthy subjects as controls. SCD blood samples were obtained via venipuncture from people with SCD, as approved by Boston Children’s Hospital Institutional Review Board protocol #X08-05-0255. Control blood samples were obtained via finger stick as approved by the University of Connecticut IRB protocol #H15-048. For finger-stick sample collection, the donor’s finger was pricked using a spring-loaded lancet and the blood droplet will be wiped three to four times before collecting a sample (Figure 9). All samples were obtained and handled in

![Figure 9 Fingerstick and blood sample preparation](Reproduced, with permission, from (Yenilmez, et al., Adv Mat Tech, 2016b))
accordance with all the relevant University of Connecticut and Boston Children’s Hospital guidelines and regulations.

All samples were tested in the magnetic levitation device within 24 hours of the blood draw. Classification of SCD was based on the increase in density of some of the RBCs in the blood sample, which is not observed in control samples. This density change was induced by the addition of 10 mM SMBS to the sample solution causing dehydration under deoxygenated conditions, which results in a wider density distribution of SCD RBCs compared to controls as described previously. To quantify this change and remove the need for user interpretation, images obtained by levitating RBCs in 50 mM Gadavist (the paramagnetic medium) with 10 mM SMBS were processed to detect the distribution of levitation heights relative to the magnets. The results were then analyzed to identify a statistically significant difference between control and SCD groups. Further, hemoglobin electrophoresis testing was conducted by the clinically accepted methods to obtain quantitative measures of the relative amounts of RBCs with normal hemoglobin (%A), SCD-mutated hemoglobin (%S), and fetal hemoglobin (%F) and the results were plotted against various measured derived from the distribution of levitative RBCs to identify any correlations.

**Image processing and analysis**

Images captured with the device are processed using a custom python script running on-board. An OpenCV library is used to remove image noise. The image is also resized by averaging pixel values to a quarter of the original resolution to enable processing using limited computation and memory resources. Also, since the image is softer due to pixel averaging, there is no need to apply additional low-pass filters (to avoid local peaks caused by noise) before calculating the gradient of the pixel intensities.

Image processing starts with the detection of the magnet and capillary edges. Since both the magnet and capillary lie on the horizontal x-axis, the gradient along the vertical y-axis is used for detection. The 1st order y-gradient between each pixel is calculated and summed for each horizontal row. The peaks
values of the obtained gradient profile (sum of $y$-gradient vs. $y$) are used to detect the location of the edges. The two outer-most peaks are taken as the magnet edges; the edges immediately adjacent to these magnet edges are taken as the capillary edges. The obtained locations are checked to confirm values within the expected range using the physical dimensions. The rows outside the detected capillary edges are discarded for subsequent analysis.

For detection of cells or particles, the $x$-axis gradient is calculated and summed along horizontal rows. The capillary edges which lie along the $x$-axis are filtered in the $x$-axis gradients. The user is warned with a message if the sum of the pixel gradients falls below the set threshold value, $T$ ($T = 80$ for this study), which indicates a low particle number or out-of-focus particles. The remaining gradient data are normalized and two normal distributions are fitted to the data.

The levitation height is reported as the means of the normal curves, calculated with reference to the lower magnet edge. Confinement width is reported as $3\sigma$ (three times the standard deviation of the normal curve). Both levitation height and confinement values are converted to physical units from pixel values using the capillary size as a reference (known to be 0.7 mm). To eliminate the slight variations in the pixel/μm scale within an experiment, the average value of the scaling factors is taken for unit conversion during the postprocessing. In addition, two Gaussian curves are fit and, from the mean and $3\sigma$ (confinement width) data of each curve, the percent separation and sickliness are calculated. Percent separation is defined as the difference between mean values of two curves divided by the higher mean. Further, “sickleness” is defined as the ratio of the peak values of the fitted curves.

Based on preliminary experiments, images with low cell numbers tend to return inconclusive data (false positives and false negatives). Therefore, all images are first checked by calculating the sum of the pixel intensity gradients (the area under the Gaussian curves), which is proportional to cell count. A threshold value, $T = 80$, is set above which there is a risk of false positives and false negatives. If this value is less than the threshold value in any captured image, it is discarded and a message is displayed to the user to
retest the sample with a greater number of cells. All data shown are filtered according to the \( T = 80 \) threshold.

**Section 3.6: White blood cell cytometry**

Whole blood was obtained via fingerstick in accordance with relevant University of Connecticut guidelines and regulations. All human experiments were approved by the University of Connecticut IRB, protocol #H15-048. Informed consent was obtained from all subjects. To observe an isolated population of WBCs at a higher concentration, whole blood was diluted 1:100 in ACK cell lysis buffer and incubated for 10 min before Gd is added (25 \( \times \) 10\(^{-3}\) M final concentration). Samples were loaded into a capillary tube and levitated in the device for 10 min before imaging. Images were taken at various positions in the capillary to obtain a sampling of the cell population. This levitation region defines the region for counting WBCs in subsequent experiments. Then, whole blood was diluted in Gadavist and inserted into the device. The cells within a set region (i.e., the FOV of one image) were counted; this process will be repeated at five randomly selected regions throughout the capillary tube by shifting the sample through the magnets and capturing an image at each location. The WBCs (i.e., the particles within the levitation region defined above) were counted in each image and averaged. Given that the volume of the sample in each FOV is approximately 0.5 µL and the sample dilution (1:1000), multiplying the average number of WBCs per FOV by 2000 yields the number of cells per microliter (where the normal range is generally considered to be 3500–11000 WBCs/µL).

**Section 3.7: Market research and customer discovery**

We will interview the following key players in the market to gain valuable insight and validate important hypotheses underlying the potential commercialization of the technology:

- Hematologists as the primary users for the SCD application
- Primary care physicians, who can provide insight on tools that are useful at the POC
- Regulatory agencies and others to understand the regulations on diagnostic devices in clinical settings
- Insurance agencies, as they greatly influence technology adoption as they determine what a physician will be reimbursed for

The questions outlined in Table 1 will be utilized to guide the interviews and gain the most meaningful information.

**Table 1 Interview questions used to validate hypotheses in Aim 4**

<table>
<thead>
<tr>
<th>Interviewees</th>
<th>Questions</th>
</tr>
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<tbody>
<tr>
<td>Hematologists</td>
<td>How does that process go, from sample collection to reviewing results with patients?</td>
</tr>
<tr>
<td></td>
<td>If you need to wait for results, does the patient have to return for a second visit?</td>
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<td></td>
<td>How would you feel about a POC test to quickly determine if a blood transfusion is needed?</td>
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<td></td>
<td>If the test was simple enough (i.e., fingerstick), would you recommend your patients own a device for home monitoring? How about in a walk-in clinic, like CVS?</td>
</tr>
<tr>
<td>Primary care physicians</td>
<td>How often do you need to send samples out for testing at a lab (out of 10 patients)?</td>
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<tr>
<td></td>
<td>How often does this delay patient care (out of 10 patients)? By how long, on average?</td>
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<tr>
<td></td>
<td>Which test or categories of tests would you be most interested in performing at the POC? Why?</td>
</tr>
<tr>
<td></td>
<td>How do the POC devices you have now affect your job and how you practice medicine?</td>
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<tr>
<td></td>
<td>What do you dislike about POC testing? What could be improved?</td>
</tr>
<tr>
<td></td>
<td>What concerns would you have with new POC technologies you have never seen before?</td>
</tr>
<tr>
<td>Regulatory Agencies</td>
<td>What is the process for a physician’s office to earn a CLIA certificate?</td>
</tr>
<tr>
<td></td>
<td>How do the processes to achieve a waiver vs. compliance vs. accreditation differ?</td>
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<tr>
<td></td>
<td>Why do you think that more clinics have compliance than accreditation?</td>
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<tr>
<td></td>
<td>When certifying a particular site, what factors come into play?</td>
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<tr>
<td></td>
<td>How do you feel about waived tests coming out? Do you think they are good or bad for healthcare?</td>
</tr>
<tr>
<td>Health Insurers</td>
<td>Does the insurance company have any say in what types of doctors administer?</td>
</tr>
<tr>
<td></td>
<td>Are POC tests better or worse than lab-based tests? How do you quantify that as a company?</td>
</tr>
<tr>
<td></td>
<td>What are the motivators and barriers to covering new diagnostic tests?</td>
</tr>
<tr>
<td></td>
<td>What is the process of investigating the potential benefits of covering new technologies?</td>
</tr>
</tbody>
</table>
**Chapter 4: Aim 1: Development of alternative versions of the setup**

The first goal was to **develop both smartphone-compatible and self-contained magnetophoresis tools which are compatible with the POC**. We hypothesize that the smartphone-based and self-contained (standalone) versions of the device will be applicable to different users. The smartphone version of the device is relatively low cost because it uses the imaging and processing capabilities of the user’s own smartphone. This is particularly important in developing countries and home-based users. However, in developed countries, this presents issues with patient privacy if a doctor or practitioner has to use his or her own smartphone. Thus, we further aim to develop a self-contained version of the device with built-in imaging and processing components that does not require any peripheral equipment.

### Modeling the magnetic field distribution

The magnetic levitation component includes two 2 mm by 5 mm by 50 mm NdFeB permanent magnets which are magnetized through the 5 mm thickness. The magnets are fixed 1 mm apart with their magnetic axes aligned vertically and with the same poles facing each other. The magnetic field distribution was modeled mathematically based on the underlying mechanisms for levitation of microspheres in the microcapillary and the theoretical dependence of levitation height and time on several parameters.

The underlying mechanisms for levitation of beads were extensively described in [45], [46]; the mathematical derivations were mainly performed in [45], [46] and also covered in several other articles [38], [47], [48].

The magnetic field, \( B \), is as follows:

\[
B(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} (-1)^k \int_{y_1}^{y_2} \int_{x_1}^{x_2} \frac{[(x-x')x + (y-y')y + (z-z')z] dx' dy'}{[(x-x')^2 + (y-y')^2 + (z-z')^2]^{3/2}} \tag{1}
\]

where \( \mu_0 \) is the permeability of free space \( (1.257 \times 10^{-6} \text{ N/A}^2) \), \( M_s \) is the saturation magnetization of the magnet \( (6.36 \times 10^5 \text{ A/m}) \), and \((x_1, x_2), (y_1, y_2), (z_1, z_2)\) denote the positions of the magnets with respect to the \( x-, y-, \) and \( z-\)axes, respectively.

**The x-component**: \( B_x \) follows from Equation (1):
\[ B_x(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} (-1)^k \int_{y_1}^{y_2} \int_{x_1}^{x_2} \frac{(x-x')dx'dy'}{[(x-x')^2 + (y-y')^2 + (z-z_k)^2]^{3/2}} \]  

(1)

Integration with respect to \( x' \) gives

\[ B_x(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{m=1}^{2} (-1)^{k+m} \int_{y_1}^{y_2} dy' \int_{x_1}^{x_2} \frac{dx'}{[(x-x_m)^2 + (y-y')^2 + (z-z_k)^2]^{1/2}} \]

The remaining \( y' \) integration can be evaluated by making a change of variable to \( \alpha = y - y' \). The resulting field expression is

\[ B_x(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{m=1}^{2} (-1)^{k+m} \ln[F(x, y, z, x_m, y_1, y_2, z_k)], \]

(4)

where

\[ F(x, y, z, x_m, y_1, y_2, z_k) = \frac{(y-y_2) + [(x-x_m)^2 + (y-y_1)^2 + (z-z_k)^2]^{1/2}}{(y-y_1) + [(x-x_m)^2 + (y-y_2)^2 + (z-z_k)^2]^{1/2}} \]

**The y-component:** \( B_y \) also follows from Equation (1),

\[ B_y(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{m=1}^{2} (-1)^{k+m} \int_{y_1}^{y_2} dy' \int_{x_1}^{x_2} \frac{(y-y')dx'dy'}{[(x-x')^2 + (y-y')^2 + (z-z_k)^2]^{3/2}} \]

(5)

Integration with respect to \( y' \) gives

\[ B_y(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{m=1}^{2} (-1)^{k+m} \int_{x_1}^{x_2} dx' \int_{y_1}^{y_2} \frac{dy'}{[(x-x')^2 + (y-y_m)^2 + (z-z_k)^2]^{1/2}} \]

The remaining \( x' \) integration is evaluated using a change of variable \( \alpha = x - x' \). The resulting field expression is

\[ B_y(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{m=1}^{2} (-1)^{k+m} \ln[H(x, y, z, x_1, x_2, y_m, z_k)] \]

(7)

where

\[ H(x, y, z, x_1, x_2, y_m, z_k) = \frac{(x-x_1) + [(x-x_1)^2 + (y-y_m)^2 + (z-z_k)^2]^{1/2}}{(x-x_2) + [(x-x_2)^2 + (y-y_m)^2 + (z-z_k)^2]^{1/2}} \]

**The z-component:** \( B_z \) is given by

\[ B_z(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} (-1)^k \int_{y_1}^{y_2} \int_{x_1}^{x_2} \frac{(z-z_k)dx'dy'}{[(x-x')^2 + (y-y')^2 + (z-z_k)^2]^{3/2}} \]

(8)

The \( x' \) integration is performed using a change of variable \( \alpha = x - x' \)

\[ B_z(x, y, z) = \frac{\mu_0 M_s}{4\pi} \int_{y_1}^{y_2} \int_{x_1}^{x_2} \frac{z-z_k}{[(x-x')^2 + (y-y')^2 + (z-z_k)^2]^{3/2}} d\alpha \ dy' \]

(9)

\[ = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{n=1}^{2} (-1)^{k+n+1} (z-z_k) \int_{y_1}^{y_2} \frac{dy'}{[(y-y')^2 + (z-z_k)^2]^{1/2} (y-y')^{2+b^2}} \]
where \( b^2 = (x - x_n)^2 + (z - z_n)^2 \). The remaining \( y' \) integration is performed using a change of variable \( \gamma = y - y' \). This gives an equation for the magnetic field which acts parallel to the buoyancy force:

\[
B_z(x, y, z) = \frac{\mu_0 M_s}{4\pi} \sum_{k=1}^{2} \sum_{n=1}^{2} \sum_{m=1}^{2} (-1)^{k+n+m} \tan^{-1}\left(\frac{(x-x_n)(y-y_m)}{(z-z_k)}\right) g(x, y, z; x_n, y_m, z_k).
\] (10)

Equations 4, 7, and 10 were solved to plot magnetic field distribution. This magnet configuration creates a magnetic field which is strongest near the magnets and approaches zero at the midline between them (Figure 10b-c). When the sample is filled into a 0.98 mm by 0.98 mm square glass microcapillary tube and inserted between the magnets, microspheres simultaneously experience a magnetic force acting in the direction of the centerline between two magnets where the magnetic flux density is minimal (Figure 10b) per Equation 10 due to the difference in magnetic susceptibility between the paramagnetic suspending solution.

Because the diamagnetic particles are suspended in a paramagnetic solution, in response to this magnetic field distribution, there is a magnetic force acting on the particles, \( F_m \) (Figure 10a). This phenomenon is referred to as negative magnetophoresis[49] and, according to this phenomenon, \( F_m \) can be expressed as follows:

\[
F_m = \mu_0 V \left[ (M_p - M_f) \cdot \vec{\nabla} \right] \vec{H} = \frac{\pi \mu_0 D_p^3}{6} \left[ (M_p - M_f) \cdot \vec{\nabla} \right] \vec{H}
\] (11)

where \( \mu_0 \) is the permeability of free space, \( V \) is the volume of the particle, \( M_p \) and \( M_f \) are the magnetizations of the particle (close to zero) and the magnetic liquid, respectively, and \( H \) is the magnetic field strength.

A buoyancy force also acts on the particles due to the density difference between the microspheres and the suspending medium, \( F_g \) (Figure 10a). This force can be expressed as follows:

\[
F_g = \frac{\pi D_p^3}{6} (\rho_p - \rho_f) \vec{g}
\] (12)

where \( g \) represents the acceleration due to gravity and \( \rho_p \) and \( \rho_f \) are the densities of the particle and the surrounding medium, respectively. For example, an object that is denser than the suspending medium
experiences a downward buoyancy force while an object which is less dense than the suspending medium experiences an upward buoyancy force. Thus, the object approaches equilibrium at a location in the magnetic field (herein simplified as the “height” relative to the bottom magnet) at which the magnetic force is equal and opposite to the buoyancy force. Due to drag forces, the equilibration of an object is time-dependent and equilibration time depends on the magnitude of the density and buoyancy forces (Figure 10g).

Further, based on this model, the equilibrium height, $h$, can be calculated. The equilibrium height is the position in the magnetic field at which the $y$-component of the magnetic field is equal and opposite the buoyancy force:

$$h = \frac{(\rho_{\text{particle}} - \rho_{\text{medium}}) g \mu_0 d^2 \chi_{\text{particle}} - \chi_{\text{medium}}}{(\chi_{\text{particle}} - \chi_{\text{medium}}) 4 B_0^2 + \frac{d^2}{2}}.$$  \hspace{1cm} (13)

where $g$ is the gravitational force, $d$ is the distance between the magnets, and $B_0$ is the magnetic field strength at the surface of the magnet.

Considering the dynamics of the particle levitation, the equilibrium time, $t$, is the defined as the time it takes a particle in the magnetic field to move from its initial position, $z_i$, to its equilibrium position, $z_f$. This is calculated by assuming acceleration is equal to zero and the sum of the magnetic ($F_m$), gravitational ($F_g$), and drag ($F_d$) forces is equal to zero: $F_m + F_g + F_d = 0$. Substituting the $y$-components of the magnetic field in Equation (1) and integrating the resulting equation, $t$ can be calculated as follows:

$$t = \frac{1}{\xi} \ln \left( \frac{\xi + \zeta}{\xi + \zeta} \right) + \zeta,$$  \hspace{1cm} (14)

where

$$\zeta = -\frac{2}{9} \frac{R^2}{\eta} \left( (\rho_{\text{particle}} - \rho_{\text{medium}}) g + \frac{2B_0^2}{\mu_0 d^2} (\chi_{\text{particle}} - \chi_{\text{medium}}) \right)$$

and

$$\xi = \frac{8}{9} \frac{R^2 B_0^2}{\mu_0 d^2 \eta} (\chi_{\text{particle}} - \chi_{\text{medium}})$$

and $R$ represents the particle radius and $\eta$ representing the dynamic viscosity of the medium. The full derivation of this conclusion can be found in[46].

It should also be noted that a magnetic force also acts in the X-direction (the axis along which the image
is captured) toward the centerline of the magnets. This causes particles to levitate along a narrow Y–Z focal plane. Thus, the optical components are adjusted such that this plane is focused by the lens onto the camera detector, allowing in-focus imaging of the particles once they reach this plane. Images of particles immediately after insertion in the magnetic field will be seen out of focus as they are randomly distributed in the X-direction and, as the depth of field is limited by the optics, many particles are out of focus. However, as the particles move toward a single focal plane, individual particles may be resolved clearly.

Further, the combined magnetic field strength generated by the permanent magnets at the back surface of the smartphone is 8 mT (Figure 10d-f), which does not cause any noticeable interference with the proper function of the smartphone. Similarly, modeling the magnetic field on the camera board, which is the closest component susceptible to the magnetic field, was modeled mathematically, the greatest magnitude was approximately 1.4 mT. As in the smartphone setup, no malfunction of electronic components was observed through the duration of these experiments.
Figure 10 Theoretical demonstration of density-based magnetic levitation

(a) Representative microspheres suspended in a paramagnetic medium for two cases: when the microspheres are more dense than the suspending medium (left) and when the microspheres are less dense than the suspending medium (right). The magnetic force ($F_m$), buoyancy or corrected gravitational force ($F_{g'}$), and drag force ($F_d$) causes the microspheres to approach equilibrium (purple dotted line). $F_m$ exerts a force on the microspheres in the direction of the centerline between the magnets (orange dotted line) and varies in magnitude depending on the microsphere’s location in the magnetic field. $F_{g'}$ exerts either a downward force (for microspheres that are denser than the suspending medium, left) or an upward force (for microspheres that are less dense than the suspending medium, right). $F_d$ acts on the object in a direction opposite the direction of motion until the bead reaches a line of equilibrium below the centerline (as in the case of denser objects, left) or above the centerline (as in the case of less dense objects, right). At equilibrium, $F_m$ and $F_{g'}$ are equal and opposite and $F_d$ has zero magnitude. (b) The magnetic field in the cross-section between the magnets demonstrated by the magnetic forces exerted on an object in the field; the forces have directionality toward the centerline between the two magnets and magnitude greatest near the magnets’ surfaces and approaching zero at the centerline. (c) Contour plot of magnitude of the magnetic field strength in the cross-sectional area at $z = 0$, the center between the two magnets. The magnitude of the magnetic field is constrained between 0 T and 0.4 T. (d-f) Contour plots showing the magnitude of the magnetic field at the back surface of the smartphone in the (d) $x$-direction, (e) $y$-direction, and (f) $z$-direction. (g) Representative images of polymer microspheres in a 50 mM gadolinium solution levitating and focusing to an equilibrium height over 120 seconds.

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Section 4.1: Goal 1.1: Smartphone-compatible setup

We first developed the smartphone-compatible setup for single-cell manipulation, imaging, and quantitative analysis. These results were presented in *PLoS ONE* [40].

Characterization of Imaging Capabilities

Sharpness was measured for 100 microspheres across the FOV and plotted as a function of their distance from the center (*Figure 11b*). Linear regression analysis shows an approximately linear correlation with sharpness decreasing as the distance from the center increases. The linear approximation for sharpness as well as the vertical image distortions and background illumination are plotted as a function of the horizontal position in the FOV (*Figure 11a*). This data shows degradation of the image quality as quantified as an increase in vertical image distortion and a decrease in background illumination and sharpness toward the edge of the FOV. The resolution of the optical setup is demonstrated qualitatively by images of 210 and 5.35 μm microspheres (*Figure 11c* and *d*, respectively), showing degradation in resolution when magnifying the image to view the smaller microspheres. However, the platform is still able to detect micro-objects of this size and determine the levitation height of a levitated and confined group of these microspheres.
Smartphone Application

The smartphone application, named Density Tester, offers a user-friendly interface for density estimation on any Android device (Figure 12a). The application prompts the user to take a photo or to choose one from the gallery (Figure 12b). The image is then retrieved and displayed on the main activity screen (Figure 12c). When the user selects ‘Process,’ the application crops the input image to the in-focus region

Figure 11 Quantitative optical characterization of the smartphone-compatible setup
(a) Quantification of image distortion (blue), background illumination (magenta), and microsphere sharpness (red) along the horizontal FOV. Microsphere sharpness is shown as the line of best fit for the data shown in (b). (b) Data points representing sharpness of microspheres located at different distances from the center of the FOV. The line of best fit approximates the decrease in image sharpness as the distance from the centerline increases. Red data points represent microspheres located to the left of the centerline and blue data points represent microspheres located to the right of the centerline. (c) 210 μm and 5.25 μm diameter microspheres demonstrating qualitatively the optical resolution.
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and detects the limits of confinement, the microcapillary edges, and the magnet edges, which are marked for the user to verify proper analysis (Figure 12d). For each image analyzed, the application displays the Gaussian fit corresponding to the pixels in the region of confinement as well as the levitation height and the standard deviation at the top of the screen (Figure 12e). The application was used to plot the dependence of levitation height on the gadolinium concentration in the suspending medium to demonstrate the accuracy and reliability of the automated analysis (Figure 12f).

![Figure 12](image)

**Figure 12** Android-based application running on the same smartphone

(a) Density Tester application icon. (b) In the smartphone application, the user is given the option to take an image using the camera application or to choose an existing image from the gallery. The selection of ‘Choose from gallery’ enables access and processing of the images stored in the smartphone or memory card installed on the smartphone. ‘Take photo’ directs the user to the camera application. (c) Once an image is taken or chosen from memory, it is loaded into the application. (d) When the user selects ‘Process’, the application performs an analysis to crop the image to the useful FOV and identify the magnet edges (blue), capillary edges (red), and microsphere confinement area (green). It also calculates the Levitation Height and Standard Deviation of the microsphere confinement area as the distance from the bottom magnet (shown as the top magnet in these images) and width of the microsphere distribution, respectively. (e) Screenshots showing the analysis results for microspheres levitated in different concentrations of gadolinium: 25 mM, 50 mM, 100 mM, and 200 mM. (f) Summary of the results obtained in (e), demonstrating the ability of the application to accurately and repeatably detect different levitation heights as a function of the gadolinium concentration in the paramagnetic medium, showing a positive correlation (n = 6).

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**Thermal Distribution in the Setup**

Due to heat generation from the smartphone and the LED during the assay, it is possible that excess heat reaches the sample in the microcapillary. This may affect the density estimation results, as an increase in temperature may cause a decrease in the density of the medium and affect the sample levitation. Therefore, the thermal characteristics of the magnetic levitation platform were quantified experimentally at the locations along the back surface of the smartphone shown in Figure 13a. The measurements indicate that all temperatures increased from room temperature (25.6 ±1°C) to a temperature between 29°C and 31°C after 2 hours (Figure 13b). Thermal measurements of the gadolinium sample and the microcapillary indicate that thermal release from the smartphone did not have a significant effect on the temperature of the sample and the microcapillary, which can be partly attributed to the shell of air between the capillary and the attachment and its potential insulation role (the thermal conductivity of air/resin is ~10⁻¹). This implies that thermal fluctuations in the sample due to the use of the smartphone will not significantly alter the temperature or interfere with the density determination.

**Figure 13** Thermal distribution within the setup

(a) Temperatures at several locations at the back surface of the smartphone were measured while the smartphone density estimation application was running. Location 8 corresponds to the back surface of the phone closest to the end of the microcapillary. Location 9 corresponds to the surface of the smartphone camera. Temperature readings over 120 minutes at locations 5, 8, and 9 at running times = 2, 5, 10, 20, 30, 40, 60, 90, and 120 minutes (n = 6). (c) Temperature in the gadolinium solution and capillary as a function of running time with standard deviation (n = 6). Reproduced from (Knowlton et al., PLOS ONE, 2015), licensed under CC BY
Experimental demonstration of magnetophoresis

The levitation height of the microspheres in the magnetic field is shown to depend on the concentration of gadolinium in the suspending medium. Figure 14 demonstrates the magnetic levitation of microspheres with time-lapse images. The graphs in Figure 14a show the upper and lower limits of confinement of the set of microspheres over time as they approach equilibrium. The width between the upper and lower limits of confinement over time was approximated by an exponential decay equation and used to determine the time to equilibrium as the time for the sample to reach a confinement width of 1.5 times the diameter of a single microsphere. Figure 14b shows that there is a positive correlation between gadolinium concentration and equilibrium height. The greatest difference in equilibrium height is seen at lower concentrations of gadolinium while higher concentrations of gadolinium cause the microspheres to approach a maximum equilibrium height near the centerline between the two magnets. This is due to the fact that the microspheres tested are denser than the suspending medium, so the buoyancy force always acts in the downward direction. At the highest concentration of gadolinium, the maximum levitation height must remain below the centerline where the magnetic force is upward in order to attain equilibrium, restricting the levitation to the centerline and below. Figure 14b also shows the negative correlation between the time to equilibrium and the paramagnetic medium concentration, with more significant differences in equilibrium time observed at lower gadolinium concentrations. All cases reached equilibrium within 6 minutes within the magnetic field and in under 1 minute for the highest gadolinium concentration tested. This is due to the higher magnetic force of higher gadolinium concentrations acting on the levitating object as it moves toward equilibrium.

Figure 14c demonstrates the focusing of microspheres with different diameters over time. The time-dependent confinement of the microspheres as they approach equilibrium was quantified as the width of confinement versus time and approximated by an exponential decay equation (n = 6 data shown in Figure 14c, right). In this way, the time to equilibrium was quantified as a function of microsphere diameter between 5 and 20 μm (Figure 14d). Larger microspheres (up to 210 μm) were also tested and shown to
reach equilibrium more rapidly than smaller microspheres (data not shown due to the high degree of user error associated with low equilibration times). This finding is consistent with Equation 13.

Finally, the relationship between microsphere density and equilibrium levitation height was characterized using a range of eight density standard microspheres ranging from 0.96 to 1.13 g/cc suspended in five different Gd concentrations between 12.5 and 200 mM (Figure 14e and f). An approximately linear inverse relationship is seen between microsphere density and levitation height as predicted by Equation 13. The difference in levitation height relative to density is greatest when suspended in medium with lower Gd concentrations, giving the greatest resolution in the range of densities tested. At higher Gd concentrations, the microspheres levitated across a smaller vertical range and near the centerline. The trend lines intersect around a density of 1 g/cc near the centerline (Figure 14e). This point gives an approximation of the density of the suspending medium. At this density, there is a weak buoyancy force due to the minimal density difference between the sample and medium; this allows the microspheres to levitate toward the low magnetic force at the centerline of the magnetic field. This data provides a calibration curve with which one may determine an unknown density given an observable levitation height and a known concentration of gadolinium in the suspending medium.
Figure 14 Experimental demonstration of magnetic levitation of polymer microspheres (a) Time-dependent focusing of 10 μm polystyrene microspheres in 30 and 100 mM Gd. Images shown at 0, 15 and 75 seconds after being placed in the magnetic field. Graphs show the top (blue) and bottom (red) confinement limits of the microspheres over time. (b) Quantification of equilibrium time (red) and equilibrium height (blue) for several Gd concentrations. (c) Time-dependent focusing of microspheres with different diameters in 50 mM Gd at time points following insertion into the magnetic field. Graphs show the width of microsphere confinement over time for 6 trials with exponential decay approximations. (d) Quantification of equilibrium time (red) and height (blue) as functions of microsphere size. (e) Calibration of levitation height to density using eight density standard microspheres in five Gd concentrations. (f) Images representing different levitation heights of different-density microspheres in two Gd concentrations. Scale bars are 100 μm.

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Section 4.2: Goal 1.2: Self-contained setup

Based on a desire for a self-contained version of the device expressed by clinicians in the US (as the use of personal smartphones introduces privacy and health concerns), we also developed a standalone version that contains its own camera and processing unit and compared it to the smartphone-based version developed in Goal 1.1. These results were presented in *Advanced Materials Technologies*[41].

Characterization of imaging capabilities

The uniformity of illumination was quantified across the FOV (Figure 15a). Pixel intensity and variation are measured as a function of the Z-position (as illustrated in Figure 15d). The average intensity represents the mean pixel intensity in vertical slices, showing that the brightness is approximately equal between the left and the right of the FOV (within 5%). Further, the variation represents the standard deviation in pixel intensities within vertical slices, demonstrating low variance from top to bottom between the magnets (±15%).

The distortion of the captured image is also quantified across the FOV from left to right (Figure 15b). As the image is distorted by compression at the edges of the FOV, the distance between the straight edges of the two magnets is plotted across the Z-axis (defined in Figure 15d) as a percent decrease relative to the same measurement at the center of the FOV. The distortion is below 3% across the entire FOV.

Bead sharpness was also quantified as the maximum pixel-to-pixel change in intensity along a profile line radiating from the center of the microsphere (Figure 15c). The bead sharpness was plotted as a function of the horizontal distance of the bead from the center (as defined in Figure 15d). The average bead sharpness is within one standard deviation in the center 75% of the FOV, with only a slight decrease in sharpness at the edge of the FOV. Microspheres could be resolved across the entire FOV.
Figure 15 Characterization of image quality in self-contained setup

(a) Uniformity of illumination within the FOV. Average intensity represents the average brightness, or pixel intensity, within 1-pixel wide vertical slices across the FOV in the area between the magnets (with no sample present). The variation represents the standard deviation of pixel intensities in 100-pixel wide vertical bins between the two magnets. 

(b) Distortion in the FOV, as measured by the vertical distance between the two straight magnet edges. All measurements are expressed as a percentage decrease in distance between the magnets relative to the average measurement in a 100-pixel slice about the center of the FOV. 

(c) Bead sharpness as a function of the horizontal distance of the bead from the center of the FOV. Sharpness is defined as the greatest pixel-to-pixel change in intensity along a profile drawn from the center of a microsphere to a point outside the microsphere. 

(d) Representative image of the imaging FOV, showing the magnetic levitation of 10 μm microspheres.

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These results indicate that the platform presented here offers a greater uniformity of illumination and particle sharpness and experiences less distortion at the edge of the FOV compared to the smartphone-compatible device (Figure 11). While the smartphone camera used in the previous setup (the Samsung Galaxy S4 did have a higher resolution (13 Megapixels compared to the 5 Megapixels used here), the images in the smartphone-compatible setup are not limited by resolution but rather are limited by the range of focal distances possible in the built-in camera. The lens in this device could be separated from the camera board at an ideal position to achieve optimal magnification and imaging quality. Further, most smartphones available on the market do not offer manual focusing capabilities; images are limited by the software's autofocusing capabilities (with few exceptions in high-cost smartphones). In contrast, this device includes a focusing lever with which the user may manually adjust the position of the lens to obtain the optimal focus. Increased image quality offered by this device compared to smartphone-compatible devices may ultimately improve the ability to image small particles and, therefore, maximize the sensitivity and accuracy of medical diagnostics.

### Experimental demonstration of magnetophoresis

Density-controlled microspheres were used to calibrate particle density to levitation height (Figure 16a and b). The result shows that the levitation height (relative to the bottom magnet) and the density of the microspheres are inversely related and the relationship is approximately linear. These linear approximations may be used as calibration curves to estimate the density of any particle from its levitation height given a known Gd concentration. Based on the theoretical model, equilibrium height, \( h \), is linearly dependent on the density of the particle as described in Equation 13. Also, the distance between \( h \) and the magnet mid-plane is inversely proportional to the concentration of Gd in the suspending medium. The approximately linear calibration curves obtained experimentally are consistent with the theoretical model.

Higher concentrations of Gd tested allow for measurement of a larger range of microsphere densities. On the other hand, smaller concentrations of Gd offer a greater difference in levitation height for smaller
differences in density; that is, smaller Gd concentrations offer a greater resolution of density detection. However, the high-density beads levitated with low concentrations of Gd were observed to settle to the bottom of the capillary because the magnitude of the buoyant force was significantly greater than the magnetic force under these conditions, indicating that low Gd concentrations result in a limited range of detection. This result is also consistent with the theoretical model. The experimental error in levitation height in each of the six data points tested was within 0.039 mm at 12.5 mM Gd and 0.026 mm at 200 mM Gd. This corresponds to an error of 0.007 g/cc at 12.5 mM Gd and 0.037 g/cc at 200 mM Gd.

Figure 16c demonstrates the ability to separate particles of different densities and identify their respective levitation heights and standard deviations using the image processing algorithm.

These forces cause a nonlinear equilibration of the particles over time (Figure 16d-i) as observed in the smartphone-compatible setup. Briefly, the concentration of Gd in the paramagnetic medium directly affects the time to equilibrium (Figure 16d-f). The upper and lower limits of the particles are tracked over time (Figure 16d and e). The equilibrium time is defined as the point at which the distance between the upper and lower limits reaches 20 μm. Higher concentrations of Gd increase the magnitude of the magnetic force and, therefore, both shifts the levitation height at equilibrium toward the centerline, which is consistent with Equation 13 and decreases the time for a particle to reach equilibrium, which is consistent with Equation 14 (Figure 16f). Testing of a range of different size microspheres demonstrates that the equilibrium levitation height of the particles is independent of particle size (Figure 16g-i); this result agrees with the analytical model in Equation 13. However, the time to equilibrium is inversely related to the microsphere size, which is also consistent with the analytical model in Equation 14.
Figure 16 Self-contained magnetic levitation platform for magnetophoresis.

(a) Linear calibration curves correlating microsphere density to levitation height for five Gd concentrations (12.5–200 mM). (b) Levitation of different density microspheres in varying concentrations of Gd (images enhanced for visibility). (c) Imaging and processing of the levitation of a mixture of two different density microspheres over 2 min. (d) Representative images demonstrating the effect of Gd concentration on levitation height and time to equilibrium for 10 μm particles. (e) Upper and lower limits of particle levitation over time. (f) Quantification of the effect of Gd concentration on equilibrium time (red) and levitation height at equilibrium (blue). (g) Representative images demonstrating the effect of particle size on levitation height and time to equilibrium in 50 mM Gd. (h) Upper and lower limits of particle levitation over time. Data points are averages over five trials with error bars representing the standard deviations. (i) Quantification of the effect of particle size on equilibrium time (red) and levitation height at equilibrium (blue).

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Comparison of the two versions of the magnetophoresis-based tool

We have demonstrated a digital and lightweight platform to measure densities of micro-objects, which employs permanent magnets fixed with like poles facing each other, a disposable microcapillary, optical components, and a custom-developed Android application running on a smartphone or a built-in imaging and processing system. The presented platforms do not require a dedicated microscope for small-scale imaging, antibody labels to distinguish particles, or complex assay protocols for reliable diagnostic testing.

This small-scale method has been validated with densities of 0.96–1.09 g/cm³. Increasing the density of the paramagnetic medium by using other solvents such as a saturated salt solution (ρ = 1.204 g/mL) or chloroform (ρ = 1.4788 g/mL) would shift this measurable range toward higher density particles with densities in the range of these mediums. Similarly, a lower density medium such as ethanol (ρ = 0.789 g/mL) or hexane (ρ = 0.659 g/mL) would allow measurement of a lower range of densities. Based on the experimental results, increasing the gadolinium concentration increases the range of detection because the stronger magnetic forces can overcome greater buoyancy forces associated with much more or less dense levitating objects. On the other hand, decreasing the concentration of gadolinium in the medium improves the resolution of detection (i.e., the ability to distinguish between smaller density differences among two levitating objects) but limits the range of detection (as higher and lower densities outside the range levitate against the top and bottom edges of the capillary).

Cost considerations are important in the design of POC devices, particularly for low-resource settings. The total cost of a single self-contained magnetic levitation platform is below $100, which is lower than other available POC technologies (Table 2). The majority of the cost associated with this device is due to the presence of a processing board, camera, and TFT display (about 75% of the cost of the device). However, this includes the cost associated with the necessary imaging and processing, which is critical for the use of magnetic levitation technology in POC settings. With the smartphone-compatible device, the imaging and processing are performed by a user-provided smartphone. While the user may
already own a smartphone, it is unlikely that, in clinical settings, a clinician would be able to use their personal device considering privacy concerns. Purchasing this device (such as the Samsung Galaxy S4 which was used here) adds an additional cost which is generally over $100, thus exceeding the cost of the self-contained device presented here. Therefore, by forgoing the unnecessary yet costly features contained in a smartphone, the device presented here can be obtained at a lower total cost than a smartphone-compatible device including the smartphone. Moreover, as the greatest costs are due to the use of commercially available products, the cost may be further reduced by custom designing and mass manufacturing the components for this specific purpose.

Table 2 Cost analysis of components in the self-contained device (*electronics for imaging and image processing)

<table>
<thead>
<tr>
<th>Self-contained Device components</th>
<th>Estimated cost (per device)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3D printed casing (raw materials only)</td>
<td>$5</td>
</tr>
<tr>
<td>NdFeB magnets (2)</td>
<td>$6.32</td>
</tr>
<tr>
<td>LED illumination</td>
<td>$5</td>
</tr>
<tr>
<td>Raspberry Pi model A+</td>
<td>$24.95*</td>
</tr>
<tr>
<td>Adafruit PiTFT 2.2” TFT</td>
<td>$24.95*</td>
</tr>
<tr>
<td>Raspberry Pi camera board</td>
<td>$19.95*</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$86.17</strong></td>
</tr>
</tbody>
</table>

Further, although the smartphone-attachable magnetic levitation device is portable and inexpensive, making it promising how resource-limited settings, potential difficulties in standardization via different smart-phone brands or hardware, operating systems (iOS, Android, Symbian, RIM, etc.), and/or different versions of the same brands may cause an inconvenience for health technicians, doctors, or patients. In contrast, the patient data can be transferred from the presented self-contained device via Bluetooth (not presented here) and/or a cable to a smartphone or computer. Therefore, a variation in the hardware or software of the local user's device will not affect the quality of diagnosis and monitoring of disease, but such internet-connected devices can be used for data transfer and communication if necessary.
Hence, while the smartphone-compatible version may be best suited to use in limited-resource settings or at home, where the user would use his or her own smartphone, the self-contained version would be more applicable to clinical applications in developed countries.

Due to the small size scale of the platform, there is an upper limit to the object which may fit in the microcapillary tube with an inner dimension of 700 μm and which may be detected with precision by the detection algorithm—we estimate this dimension to be around 210 μm. There is also a lower limit to the object size which may be imaged by the camera used here. This dimension is also particularly limited by the autofocusing feature built into the camera (as only a few smartphone models currently available offer manual control over the focus). Another limitation on this dimension is the high focusing time for smaller particle diameters: particles of 5.35 μm diameter took over 6 minutes to reach equilibrium. An absolute theoretical lower limit to microsphere size exists due to Brownian motion causing very small particles to remain evenly dispersed in the medium.
Chapter 5: Aim 2: Extension of the capabilities of the device

Section 5.1: Goal 2.1: Flow-assisted dynamic magnetophoresis

Because some cell types, such as CTCs, occur at a very low frequency in human peripheral blood (a diagnostic indicator is generally considered >5 CTCs/mL of blood), it is necessary to “search” for CTCs in a larger sample. Thus, it was proposed to flow a sample through the magnetic field, continuously record images as they pass through the FOV, and identify cells from this series of images. A microcontroller receives the set points selected by the user for flow rate through a piezoelectric micro-pump flow (in terms of input amplitude and frequency of the micro-pump) and LED intensity. Then, it generates the appropriate outputs for the micro-pump controller and LED in order to achieve the set points. Further, a smartphone application is used to control the system and image cells as they flow through and analyze the obtained videos to detect the number of particles in each density (i.e., levitation height) range. These results were presented in RSC Advances[42].

Theoretical modeling

First, the magnetic levitation phenomenon under flow conditions is considered and illustrated schematically in Figure 17: The magnetic field generated by the two permanent magnets is the same as described in the static setup. The magnetic focusing of particles suspended in a paramagnetic medium and flowing through the magnetic field can be described as a combination of four forces. The gravitational force ($F_g$) is due to the object's buoyancy, or density relative to that of the medium. This force is upward in the case of a particle less dense than the medium and downward in the case of a particle denser than the medium. The magnetic force ($F_m$) is due to the object's low magnetic susceptibility relative to the medium with directionality toward the center between the magnets. This force is strongest near the surface of the magnets and approaches zero at the center line between the magnets. Thus, in the static case (no flow), a particle will move vertically to find equilibrium between $F_g$ and $F_m$. As the particle moves toward equilibrium, it experiences a drag force ($F_d$) opposing the motion of the particle. Finally, in the case where pressure is applied to drive fluid flow, shear force ($F_s$) is also present, acting in the horizontal direction.
Since the flow rate dictates the time that particles remain in the magnetic field and affects the confinement rate of particles, the effect of flow rate has been investigated. 10 μm polystyrene microspheres were suspended in 50 mM Gd. The solution was pumped between the permanent magnets in the magnetic focusing module with flow rates of 0.1, 0.25 and 0.75 μL/s. Since the length of the magnet and flow rate determine the magnetic forces and exposure time to these forces by micro-objects, the flow experiments have been performed for a range of magnet lengths: 10, 20, 30, 40 and 50 mm.

**Effects of flow rate and magnet length**

**Figure 18a-c** shows the distribution of 10 μm polystyrene microspheres suspended in a 50 mM Gadavist solution and pumped at different flow rates. At a 0.1 μL/s flow rate and 50 mm magnet length, over 95% of the particles were confined to a band with a width of 14 μm (which is ±2 μm around a single microsphere size (i.e., 10 μm). As the flow rate was increased to ~0.25 μL/s and ~0.75 μL/s, the microspheres remained for a shorter time in the magnetic field (i.e., exposed to magnetic forces for a shorter time) and thus attained a wider distribution of microspheres around the equilibrium line. **Figure 18d** shows that for all magnet lengths, increasing the flow rate increases the standard deviation of microsphere's distribution (wider distribution).
Figure 18 Micro-object focusing at different flow rates and a range of magnet lengths
(a–c) Histogram of microsphere distribution within the magnetic field of 1 cm (a), 3 cm (b), and 5 cm (c) magnet length, while flow is on for 1 minute 30 seconds on average (total elapsed time stamps (T) and total counted number of particles (N) are given at the inset of each histogram). Capillary width is 700 μm (i.e., −350 represents the top of the capillary and 350 represents the bottom of the capillary in the vertical axis of histograms). At lower flow rates, the Gaussian distribution fit to the data has a slightly higher standard deviation. Similarly, at lower magnet lengths, the Gaussian distribution has a greater standard deviation than at greater magnet lengths. (d) Standard deviation of micro-object confinement versus flow rate at various magnet lengths. (e) Standard deviation of micro-object confinement versus magnet length at various flow rates.
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The results shown in Figure 18a-c indicate that 95% of the particles were confined to their equilibrium at a 0.1 μL/s flow rate and 50 mm magnet length. Decreasing the length of the magnets results in a wider distribution of microspheres around the equilibrium line. Figure 18e shows that, for all flow rates tested, increasing the magnet length decreases the standard deviation of the microsphere's distribution (more confinement). However, it should be noted that the strength of the magnetic field between the permanent magnets varies throughout the length of the magnets and is at its maximum at the two ends of magnets. Therefore, magnets should be perfectly aligned at their ends to avoid any abrupt changes in the focusing height of the particles as they leave the magnetic field.

Continuous separation during flow

To demonstrate the sorting capability of our approach for heterogeneous mixtures of particles suspended in the same sample, we first used two microspheres: one with a volumetric mass density of 0.97 g/cm³ and the other with a volumetric mass density of 1.12 g/cm³. Figure 19a-c demonstrates sorting of particles on the basis of their volumetric mass densities. At lower flow rates, e.g. 0.1 μL/s and 0.2 μL/s, the particle sorting was more efficient. Around 10% overlap between the particle distributions across the width of the capillary was observed at a flow rate of 0.1 μL/s. At the highest flow rate, the separation between the distributions was less distinct, with a 25% overlap due to less exposure to the magnetic field and, thus, magnetic forces.

Figure 19d and e demonstrate the magnetic focusing of blood samples obtained via fingerstick and the sorting of the cells from microspheres at flow rates of 0.18 μL/s (Figure 19d) and 0.05 μL/s (Figure 19e). Results show that blood cells take a longer time to equilibrate than microspheres. Sorting was achieved without overlap between distributions at 0.05 μL/s (Figure 19e). Relatively much lower numbers of microspheres compared to red blood cells in Figure 19d and e also demonstrate the rare particle detection and sorting capability of this approach.
Section 5.2: Goal 2.3: Fluorescence imaging

Fluorescence microscopy is an advanced form of microscopy offering specificity by using fluorophore-conjugated antibodies or other fluorescent stains to identify certain cell markers. In fluorescence microscopic imaging, these fluorophores are excited when an electron absorbs energy from a specific wavelength of light; when the electron returns to its ground state, light is released (with a longer wavelength due to a small amount of energy loss) and is detected by a camera. A typical fluorescence microscope involves the use of an excitation filter, a dichroic mirror, and an emission filter to achieve optimal excitation of the fluorophore while reducing the noise from the excitation light as much as

Figure 19 Sorting of blood cells and particles at low and high flow rates
(a–c) Images of the microspheres from the cellphone screen and corresponding distribution of two micro-objects of different known densities suspended in the same sample at (a) 0.1 μL/s, (b) 0.2 μL/s, and (c) 1 μL/s. The blue histogram distribution represents the focusing height of the 0.97 g cm⁻³ microspheres and the yellow histogram distribution represents the 1.12 g cm⁻³ microspheres. (d–e) Images of the microspheres from cell phone screen and blood cells, and corresponding summed and normalized pixel intensities of micro-objects and blood cells flowed at (d) 0.18 μL/s and (e) 0.05 μL/s. Relatively low numbers of microspheres compared to blood cells demonstrates the rare particle sorting capability. Reproduced from (Amin et al., RSC Adv, 2016) with permission from The Royal Society of Chemistry
possible. This technology has broad applications in many fields of medical research and clinical disease diagnostics, including molecular and cell biology research[50]–[54], cancer research[54], [55], bioluminescence imaging[56], and diagnosis of infectious diseases[57]. However, the components of a typical fluorescence microscope (excitation lasers or light filters, dichroic mirrors, and emission light filters) introduce a high cost and low portability, preventing the use of fluorescence microscopy in resource-poor areas. Fluorescence microscopy has been incorporated into several mobile phone-based diagnostic tools,[58], [59] with broad applications for imaging and sizing DNA molecules[60], quantifying WBCs by optofluidic analysis[61], detecting pathogens such as water-borne *Giardia lamblia*[62], and imaging viruses and bacteria[63], [64].

Hence, building upon the density-based cell separation approach and the imaging and processing capabilities of the smartphone, we further integrate a fluorescence imaging module. This will expand the multiplex test capabilities of this compact, low-cost, and versatile technology. The combination of two highly versatile techniques—magnetic focusing and fluorescence microscopy—are expected to result in a powerful POC-compatible tool. These results were presented in *Lab on a Chip*[43].

| Effect of microsphere size and light conditions on image quality |

Brightfield and darkfield imaging returned similar results for both conditions. The qualitative images in *Figure 20a* shows comparable bead clarity, resolution, and detection for beads ranging from 5.35 μm to 79.0 μm. Although features of the 5.35 μm beads are not clear, their visibility indicates this microscope's ability to detect small objects such as cells. Microspheres larger than 10.4 μm provide further detail in both types of images. Analysis of bead size in MATLAB for the five different sizes in both light conditions shows no significant difference between size seen in brightfield and darkfield images (*Figure 20b* and *c*). Some limitations of the size measurement method used here include subjective interpretation of the bead edges based on the pixel intensity profiles, considering both shadows and scattering of light. However, the consistency of image quality and bead size across transmitted light and reflected light conditions indicate the suitability of our design for darkfield and fluorescence imaging.
Figure 20 Brightfield and darkfield imaging results

(a) Microspheres of various sizes (5.35 μm, 10.4 μm, 20.0 μm, 40.0 μm, and 79.0 μm) imaged under brightfield (BF)(left) and darkfield (DF)(right) conditions. (b) Representative image of how bead diameters were measured in brightfield (left) and darkfield (right) images based on pixel intensity using the improfile function in MATLAB. The black, double-headed arrow shows the horizontal distance considered to be the bead diameter for the beads marked in blue. (c) Diameter of the imaged beads for all sizes and both imaging conditions tested.

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Effectiveness of fluorescence imaging

Images of fluorescent microspheres taken with the proposed device display the effectiveness of the microscope in capturing emitted wavelengths. In Figure 21, red, green, and blue fluorescent microspheres show the most contrast with the other colors using the appropriate physical channel and digital filter. For the red microspheres (Figure 21a), the green and blue filters effectively reduce the signal strength in all three digital channels. With the green filter, the red signal strength is still comparable to the green signal strength due to the proximity of these wavelengths, the intensity of the red signal, and the imperfect boundaries of the digital channels (Figure 21d). Using the red filter, the red channel's signal strength is significantly greater than the signal strength in the green or blue channels (Figure 21d). Qualitatively, the image corresponding to both the red filter and the red digital channel is the brightest of the split images and contrasts most with other filter and channel combinations (Figure 21a). The strength of the signal in the red channel with the red filters compared to that with other filters supports the hypothesis that the microsphere emits red light via fluorescence in response to white light excitation and that the filters used can effectively separate noise due to scattered white light. This allows the device to distinguish between fluorescent micro-objects emitting different colors of light.

The green microspheres (Figure 21b) also show the strongest filtered signal using the green emission filter and green digital channel. The red filter provides an attenuated signal in all three channels while the green filter maintained signal strength in both the green and blue channels (Figure 21e). This comparable signal strength between the green and blue channels using the green emission filter can be explained by both the imperfect boundaries of the camera's color sensitivity (Sony Corporation) and the fluorescent color of the microspheres. The peak emission for the green microspheres is actually approximately 500 nm (Cospheric LLC), which corresponds to blue–green light. As a result, the signals in the blue and green channels with the blue emission filter are also relatively strong compared to the signal strength for the same conditions with the red microspheres. Qualitatively, the blue-green quality of the green
microspheres can be seen in the brightness of the sample in the green channel with the green emission filter and in the blue channel for both the green and blue emission filters.

The blue microspheres show the strongest filtered signal using the blue emission filter and blue digital channel (Figure 21c). Although the blue channel shows a significantly stronger signal than the green or red channels in the composite image, the blue emission filter attenuates the green and red channels (Figure 21f). Furthermore, with the red emission filter, no emitted light is captured by the camera due to the attenuation of blue light as the filter allows only higher wavelengths (>500 nm) to pass through while greatly reducing the transmittance of light having a wavelength lower than 500 nm (Figure 21c and f).
For all three differently colored microspheres, both physical and digital filters are needed to increase the contrast between the emitted fluorescent light and scattered or excitation light in the background. The filtered images are brightest and show the strongest signal relative to the background when the same-color emission filters and digital channels are used. This finding is significant for two reasons. First, the proposed fluorescence microscope picks up the light emitted by the fluorophore. Second, the dual filtering ensures that only the desired color of light is analyzed in the processed image. Like the traditional fluorescence microscope, an area of high pixel intensity corresponds to a fluorescent micro-object emitting a desired wavelength of light. Also similar to more expensive designs, the proposed microscope can easily change the color of light picked up by switching out the physical filter and choosing a different digital channel in which the image is analyzed.

**Filter and LED optimization for cell imaging**

Given the effectiveness of the fluorescence microscope for microspheres, the device was optimized for fluorescent cell imaging using two different types of cytoplasmic stains: calcein and QTracker 625. **Figure 22a** shows the excitation and emission spectra for the stains alongside the LED spectrum, emission filter light transmission, and smartphone camera's green channel to indicate that the filtered images in the green channel and capture the calcein's emitted light while reducing noise from the blue excitation wavelengths. The images in **Figure 22b** demonstrate the effectiveness of each emission filter for blocking the blue excitation light from being captured by the smartphone camera while allowing the green light from the cells to be imaged. All three green emission filters slightly attenuated the signal strength in the green and blue digital channels while significantly attenuating that in the red channel (**Figure 22c**). Based on both the quantitative and qualitative analyses of the three green filters, the gaslight green filter was determined to be the most effective because it least attenuated the green signal. However, the similar effect of all three filters displays versatility in the design; in a low-resource setting, several types of green filters will suffice to image calcein-stained cells. With the gaslight green filter, individual cells were clear and bright in the green channel (**Figure 22d**) and were able to be counted.
digitally in ImageJ (Figure 22e). The ability to digitally count cells after simple filtering in this device increases its applications while reducing the manpower needed to analyze its results. However, if counting is done visually, the external magnetic field brings the cells into a line at equilibrium in the middle of the FOV, rendering them easier to count than if they were spread out on a glass slide under a traditional fluorescence microscope.

**Figure 22 Live cell fluorescence imaging**

(a) Wavelengths used to optimize filters for fluorescence imaging for calcein. From top to bottom: excitation and emission spectra for calcein; blue LED emission; transmittance for various green emission filters; relative absorbance of color channels in the camera. (b) Raw smartphone images of cells stained with calcein with no emission filter and gaslight green (GG), moss green (MG), and pea green (PG) emission filters. (c) Analysis of calcein fluorescence signal strength for four filter conditions and three digital channels. (d) Calcein-stained cells imaged with the GG emission filter and split into red, green, and blue channels. (e) Representative image of cell counting using the “find maxima” function with a noise tolerance of 50 in ImageJ. (f) Wavelengths used to optimize filters for fluorescence imaging for QTracker 625. From top to bottom: excitation and emission spectra for QTracker 625; blue LED emission; transmittance for various red emission filters (Rosco Laboratories); relative absorbance of color channels in the camera. (g) Raw smartphone images of cells stained with QTracker 625 for no filter and golden amber (GA), light red (LR), and fire (F) emission filters. (h) Analysis of QTracker 625 fluorescence signal strength for four filter conditions and three digital channels. (i) QTracker 625-stained cells imaged with the GA emission filter and split into red, green, and blue channels. (j) Representative image of cell counting using the “find maxima” function (noise tolerance 30) in ImageJ. Reproduced from (Knowlton et al., Lab Chip, 2017) with permission from the Royal Society of Chemistry.
Characterization of cell imaging

Cell counting (or cytometry) is another of the many possible biological applications for our device. This application was characterized using calcein-stained breast cancer cells in the proposed fluorescence microscope. Figure 23a displays the images obtained for samples of five different concentrations. Because these images have been physically filtered using gaslight green plastic colored filter and digitally filtered by splitting channels in ImageJ, the areas of brightness in the photographs correspond to areas of high-intensity green light, or to the stained cells. In Figure 23a, the cells are aligned at equilibrium due to the external magnetic field; relative concentrations of the five dilutions are evident. A quantification of the cell concentrations shows that the proposed design accurately provides relative concentration measurements through either visual or digital methods (Figure 23b). Both methods exhibit linearity ($R^2 > 0.95$) with the theoretical percentage of the hemacytometer's cell concentration determination. This reliable measurement of relative concentration in a small (10 μl) sample reduces the need to obtain, store, and analyze large volumes of biological samples and could be used to determine abnormal cell counts indicative of disease. For all five dilutions, the proposed fluorescence microscope's images underestimate the theoretical cell concentration (Figure 23b). This difference may be due to the error inherent in all three methods or due to cell death in the time elapsed between hemacytometer counting and sample imaging. While this slight underestimation presents an area for future optimization of the device, the strong correlation between measured cell concentration and true cell concentration shows promise for diagnostic capabilities of the proposed device.

The lowest cell concentration measured by this device is limited to one single cell in the smartphone camera's FOV (Figure 23c). At a zoom level typically used to capture images, this limit is 1 cell in 1.07 μL, or 935 cells/mL. However, it is possible to zoom out to a lower limit of detection of 1 cell in 1.47 μL or 680 cells/mL. The upper limit of detection for cell concentration is essentially unlimited; it is possible to dilute the sample for cells that are not resolvable due to high cell concentration. Another factor affecting cell detection is cell size. An analysis of the calcein-stained cells in a sample of A549 cells of
various sizes showed that the device can detect cells with a minor ellipse axis as low as $8.17 \, \mu m$ (Figure 23d). Thus, cytoplasmically stained cells with diameters of at least $8.17 \, \mu m$ are likely to be detected using this device. Because microspheres with diameters as low as $5 \, \mu m$ (Figure 23a) are seen in the device as well, successful staining and detection of smaller cells is an area for future optimization.

Figure 23 Cell counting and limit of detection using florescence imaging
(a) Physically and digitally filtered images of calcein-stained cells at different concentrations: 1%, 2%, 3%, 4%, and 5% of the total original cell concentration. Each double-headed blue arrow represents 0.1 mm (100 μm) of the sample. (b) Measured cell concentration using visual counting and the ‘find maxima’ function in ImageJ, both of which are compared to the theoretical cell count, determined as a percentage of the original hemacytometer count. Linear trendlines show the correlation between visual or ImageJ cell counts and the hemacytometer cell count. (c) Images of a single calcein-stained HeyA8 cell in a zoomed-in (top) and zoomed-out (bottom) FOV. (d) Two images (left) of a sample containing calcein-stained A549 cells of various sizes. The graph to the right quantifies the various cell sizes in this sample through boxplots of the length of major and minor axes of the ellipses corresponding to each cell. The “all” series is an aggregate of the major axis and minor axis lengths.

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**Cell imaging applications**

The device presented here has shown promise for a variety of applications involving the use of fluorescence microscopy to distinguish two different cell populations. In **Figure 24a**, cells stained with red QTracker 625 are much more rare than the cells stained with green calcein in the sample. The raw image with no emission filter shows the presence of both cell types, and splitting the image into green and red channels verifies that each color is indeed as it appears to the human eye. The image using the gaslight green filter again shows both cell types, which is likely due to the filter's relatively high transmission of red wavelengths, above 600 nm (**Figure 24a**). The fire filter, however, almost completely eliminates the visibility of the cells that are stained green. Due to the sensitivity of this filter, only the rare cell stained red is visible. This finding is significant because brightfield conditions would not be able to distinguish between the cells, but the fluorescence microscope with easily changeable and customizable filter sets can do so. Additionally, the magnetic field focuses these cells on a single plane such that the rare cell is in focus. Therefore, incorporation of appropriate filters and stains into this device can be utilized in identification and detection of rare cells in a sample with other, more populous cells.

The fluorescence imaging mode also has the capability to eliminate one population from the image. In **Figure 24b**, a mixture of red blood cells and calcein-stained cancer cells is imaged in both brightfield and darkfield conditions. In brightfield conditions, both populations are seen. Both populations are spatially separated in the magnetic field due to their different densities. In fluorescence imaging mode, in contrast, only the cancer cells are seen because RBC staining with calcein is not observed. The RBCs do not interfere with the appearance of the cancer cells due to the aforementioned spatial separation. In this way, the combination of magnetophoresis and fluorescence imaging can both separate and differentially image the cell populations. This may be of interest in the context of blood pathologies, such as circulating tumor cells or circulating endothelial cells[65], which are currently difficult to detect.

Lastly, the use of another stain, acridine orange, may aid in cancer detection. In **Figure 24c**, both cancer cells (A549) and non-cancerous mouse fibroblasts (3T3) are successfully stained with acridine orange and imaged
with the smartphone camera. Because there is no emission filter in these images, all wavelengths of light are collected. The image can then be split into red, green, and blue channels digitally. In this application, red and green channels are of interest due to the high RNA content—and therefore more red emission—of rapidly dividing cells such as cancer cells[44]. A comparison of the signal strength in the red and green channels for both images of similar quality shows that both types of cells have a much stronger signal in the red channel than in the green channel, and the cancer cells have a slightly higher red to green signal strength ratio than the 3T3 cells: 2.6 and 2.2, respectively. The choice to image without an emission filter proved useful, and the capability to image and analyze the acridine orange stain shows promise for this device in a point-of-care setting.

**Figure 24** Distinguishing cell populations using two-color fluorescence imaging. 
(a) Mixture of cancer cells (HeyA8) stained with calcein or QTracker as imaged under various physical emission filter and digital channel conditions. There were fewer QTracker-stained cells than calcein-stained cells. (b) Brightfield (top) and filtered, darkfield (bottom) images of a calcein-stained mixture of cancer cells (HeyA8) and blood cells. (c) Representative images (left) of lung cancer cells (A549) and mouse fibroblasts (3T3) stained with acridine orange, excited with blue light, and with no emission filter and a graph (right) displaying the signal strength of cells in the red and green channels. Reproduced from (Knowlton et al., Lab Chip, 2017) with permission from the Royal Society of Chemistry.
Cost analysis

Relying on relatively inexpensive commercially-available or 3D-printable components, the device presented here offers a low-cost alternative to traditional laboratory equipment designed for fluorescence imaging and/or cytometry. Table 3 shows a detailed cost breakdown for the device presented here. The unit cost of the device is about $100 (assuming that the user provides the smartphone) as compared with tens of thousands of dollars for fluorescence microscopes or fluorescence-activated cell sorting equipment. Each use of the device only requires replacement of the microcapillary tube and preparation of the sample in paramagnetic solution (~$1 per assay, excluding the cost of antibodies, which can vary greatly depending on the assay).

Table 3 Cost analysis of smartphone-based device for fluorescence imaging and magnetophoresis

<table>
<thead>
<tr>
<th>Component</th>
<th>Estimated cost (per device)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Device cost</strong></td>
<td></td>
</tr>
<tr>
<td>3D-printed case, material cost</td>
<td>$18.23</td>
</tr>
<tr>
<td>Lens</td>
<td>$73</td>
</tr>
<tr>
<td>Permanent magnets (2)</td>
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<tr>
<td>Battery</td>
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<td>Switch</td>
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<tr>
<td>Resistor</td>
<td>$0.10</td>
</tr>
<tr>
<td>LEDs (3)</td>
<td>$2.09</td>
</tr>
<tr>
<td>Emission filter set (includes all filters used here)</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$105.87</strong></td>
</tr>
<tr>
<td><strong>Per-use cost</strong></td>
<td></td>
</tr>
<tr>
<td>Microcapillary tubes</td>
<td>$1 per test</td>
</tr>
<tr>
<td>Gadavist</td>
<td>$17.43 per 2 mL vial, sufficient for ~4000 assays</td>
</tr>
<tr>
<td>Staining</td>
<td>Varies by application</td>
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</table>
Chapter 6: Aim 3: Demonstration of clinical applications of the POC magnetophoresis devices

The next goal was to demonstrate and validate useful clinical applications of the tools developed in Aim 1. Here, we aim to develop a platform technology—a tool that can be used for a range of applications. Thus, we propose to develop three specific clinical diagnostics applications by implementing modifications to the hardware, software, and the testing reagents used. Ultimately, this will allow a single device to be applied for a variety of diagnostics in healthcare and home settings.

Section 6.1: Goal 3.1: Sickle cell disease diagnosis

The following application was described in Scientific Reports[66] and Advanced Materials Technologies[67].

Characterization of diagnostic capabilities

To test the effectiveness of the platform to detect SCD, blood samples from control donors and donors previously diagnosed with SCD were deoxygenated and dehydrated with 10 mM sodium metabisulfite dissolved in the paramagnetic gadolinium solution throughout the experiment to induce a density increase in SS cells. When RBCs were suspended in the solution, placed in the magnetic field, and allowed to reach equilibrium (10 minutes), control cells (n = 4) were observed to levitate at a higher levitation height and within a narrower area of confinement than the SS cells (n = 11) in both the microscopy compatible setup (Figure 25a-b) and the smartphone-compatible magnetic levitation platform (Figure 25c-d). This is due to the increase in SS RBC density following sodium metabisulfite-induced deoxygenation and dehydration, which is significantly more pronounced in SS RBCs compared to control RBCs. The degree of confinement of the RBCs was calculated as the standard deviation of the RBC levitation heights; each result is the mean over six trials (Figure 25e). A non-parametric Mann-Whitney-Wilcoxon two-sided test was used to verify that the difference between distributions of the sickle cell group and the control group is significant (t approximation, n1 = 4, n2 = 11, p = 0.0181) (Figure 25f). This quantifiable and repeatable
difference indicates that this method of magnetic levitation of RBCs in sodium metabisulfite and gadolinium is a simple yet powerful binary measure for detection of SCD.

Figure 25 Distinguishing control from SCD blood based on confinement width
Levitation of (a) control and (b) SS RBCs in 50 mM Gd with 10 mM SMBS using a microscopy compatible-setup. Levitation of (c) control and (d) SS RBCs in 50 mM Gd with 10 mM SMBS using the smartphone-compatible magnetic levitation platform. (e) Distribution of Wilcoxon scores for confinement width of 4 control and 11 SS RBC samples analyzed using the smartphone-based magnetic levitation platform. Results show a statistically significant difference between the groups according to a two-tailed non-parametric Mann-Whitney-Wilcoxon test with a t-approximation (α = 0.05). Reproduced from (Knowlton et al., Sci Rep, 2015), licensed under CC BY 4.0

The SS RBCs, even in the absence of induced dehydration, levitate at slightly lower heights than control RBCs. The height difference, although observable when using bright field microscopy (20× and above using Kohler illumination), is not significant enough for observation via smartphone imaging in order to generate reliable measurements. Therefore, in order to improve reliability of the diagnostic test, increase the sensitivity and specificity of the approach, and minimize the assay time, dehydration was induced in
RBC samples, amplifying the difference between control and SS RBC levitation. These results indicate an observable and statistically significant difference in the levitation of RBCs from people with SCD compared to control samples.

To enhance the automated analysis used to distinguish SCD samples from control samples (levitated in 50 mM Gd and 10 mM SMBS), additional quantitative measures of the RBC distribution were tested. In addition to the confinement width described above (Figure 26a), which was defined based on a single fitted Gaussian curve, fitting two Gaussian curves was also tested on the basis that the blood of a person

![Figure 26](image)

**Figure 26** Other RBC distribution parameters for distinguishing control from SCD blood
(a) Levitation height and confinement width of RBCs in a magnetic field with a single normal fit. (b) Sickleness (the ratio between the normal fit heights) and separation (the difference between the average levitation heights normalized to the greater mean value). (c) Experimental results for confinement width of control (n = 48 images over four subjects) and SCD (n = 93 images over ten subjects) RBCs, defined as the total width of two Gaussian curves fit to pixel intensity gradient data. Images with a low cell number (i.e., sum of pixel intensity gradient less than 80) have been discarded from this data set. (d) Experimental results showing separation % and sickleness for the same control and SCD samples displayed in (c).

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with SCD contains different subpopulations of RBCs as described above. Based on these two Gaussian curves, the percent separation was defined as the difference between mean values of two curves divided by the higher mean and “sickness” was defined as ratio of the peak values of the fits (Figure 26b). Results for the same data set used in Figure 26c demonstrate that using a combination of these two measures, SCD samples can be better distinguished from control samples as shown in Figure 26d.

**Robustness with sample storage time**

In order to be used in a clinical setting, the test results should be consistent over time in storage in the case that a sample cannot be analyzed immediately. Three SCD samples were stored at 4°C in vacutainers with EDTA added to prevent clotting and analyzed over 8 days. The levitation height (Figure 27a) and confinement width (Figure 27b) results are consistent over 8 d, with each data point falling within $3\sigma$ of the average as observed qualitatively in Figure 27c.

![Figure 27](image_url)

**Figure 27** Testing of SCD RBCs taken over eight days after the blood was drawn (a) Levitation height and (b) confinement width of three SCD subjects over eight days after sample collection and stored at 4°C. (c) Representative images showing SCD RBCs at equilibrium, taken up to eight days after sample collection and stored at 4°C (day 0 is the day samples were collected).

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Comparison with existing technologies

Compared to existing complex technologies like density-based centrifugal separation[10], solubility testing based on Hb aggregation[11], and non-electrolyte hemolysis[12], the proposed platform does not require any peripheral equipment and is relatively inexpensive (less than $100 compared to several hundred dollars). Thus, unlike the other methods, it is feasible to have this tool at the POC. Further, there is a paper-based chromatography technique[13] that can also diagnose SCD without peripheral equipment. However, the cost per test is $5, compared to the $1 cost of the microcapillary, gadolinium, and SMBS required for this test; thus, the test proposed here is more feasible for limited-resource applications. Further, this method only requires about 10-15 minutes, thus providing results while the patient is still in the office, which is not possible with many other techniques. Finally, other approaches require a certain degree of technical skill for the sample preparation or have analog readouts that must be interpreted by a trained user. In contrast, this platform provides a simple user interface that walks the user through the straightforward sample preparation and no prior knowledge is needed to execute the test.

Table 4 Comparison of the magnetic levitation platform to other technologies for SCD diagnostics

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<tr>
<td><strong>Required equipment</strong></td>
<td>Compact, handheld device</td>
<td>Centrifuge</td>
<td>Incubator, electrophoresis system</td>
<td>Tonometer, water bath, centrifuge, microscope</td>
<td>None</td>
</tr>
<tr>
<td><strong>Equipment cost</strong></td>
<td>$&lt; 100</td>
<td>$&gt; 300</td>
<td>$&gt; 500</td>
<td>$&gt; 700</td>
<td>-</td>
</tr>
<tr>
<td><strong>Cost per test</strong></td>
<td>$&lt; 1 (microcapillary, Gd, SMBS)</td>
<td>$&lt; 1 (capillary, multiphase system)</td>
<td>$&lt; 1 (cellulose acetate strips, SMBS)</td>
<td>$&lt; 1 (isosmotic hemolysis solution)</td>
<td>$&lt; 5 (patterned paper, Sickledex solution)</td>
</tr>
<tr>
<td><strong>Doctor/technician skill</strong></td>
<td>Simple user interface, straightforward sample loading, no knowledge necessary</td>
<td>Simple sample preparation, centrifuge requires trained technician</td>
<td>Procedure is tedious and requires trained technician</td>
<td>Procedure is tedious and requires trained technician</td>
<td>Samples are differentiated visually by a trained technician</td>
</tr>
<tr>
<td><strong>Time</strong></td>
<td>10-15 min</td>
<td>10-15 min</td>
<td>24 hrs</td>
<td>90 min</td>
<td>20 min</td>
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</table>
Potential for SCD monitoring

Furthermore, there is potential for this device to be used to monitor the status of SCD patients. The %HbS value is a measure of the relative amount of the mutated form of hemoglobin in the blood. One common treatment for SCD is a drug called hydroxyurea, which increases the level of the fetal form of hemoglobin (HbF), which does not exhibit the same sickling effect; as a result, this treatment also decreases the %HbS value. For cases in which hydroxyurea has not been effective, another common treatment is periodic transfusions to supplement the blood with the normal form of hemoglobin, thereby, similarly, decreasing the %HbS value. In both such cases, physicians generally utilize the %HbS value as a metric for monitoring a patient over time and determining the proper course of treatment (e.g., adjusting hydroxyurea dosage or conducting a blood transfusion). However, the %HbF and %HbS are generally measured by drawing a vial of blood and conducting hemoglobin electrophoresis. This generally takes a few days, meaning that a doctor does not have enough information to treat the patient at regular checkups unless the patient visits the diagnostics laboratory beforehand. Thus, it would be extremely useful to have a rapid and portable tool to conduct the same measures while a patient is in the office or even at home.

Thus, to find a correlation between the magnetic levitation distribution and the %HbS or %HbF, which would indicate that magnetic levitation can be used as a predictor for a SCD patient’s status over time. The results are shown in Figure 28; each data point represents at least nine measures from a single patient and four samples were discarded because there were very few cells in the FOV and, thus, a high variation between measures. Three different measures were tested: sickleness, percent separation, and confinement width (as defined in Figure 26). However, while the observed trends were consistent with the expected correlations, the correlations between these measures and %HbF and %HbS were very weak. Thus, given the available data, no conclusions can be made at this time regarding the clinical utility of the proposed platform for monitoring SCD status over time. Further work must be done, such as testing other RBC distribution parameters or testing additional samples.
Figure 28 Investigation of the potential for SCD monitoring

(a) Sickleness, (b) percent separation, and (c) confinement width as functions of the percent HbF measured by hemoglobin electrophoresis and the line of best fit. (d) Sickleness, (e) percent separation, and (f) confinement width as functions of the percent HbS measured by hemoglobin electrophoresis and the line of best fit.
Section 6.2: Goal 3.2: White blood cell (WBC) quantification

The following application was described in *Advanced Materials Technologies*[41].

Demonstration of WBC cytometry

Error! Reference source not found. shows a useful application of this platform to levitate and isolate WBCs in blood for rapid, automated cytometry. In Error! Reference source not found.a, the region of levitation for the WBC population can be identified by lysing the RBC population with ACK buffer and levitating the sample (the pictured sample has been concentrated for better visualization), thus defining the region for WBC counting in subsequent experiments in the presence of RBCs.

Error! Reference source not found.b demonstrates a method of sampling to count WBCs within the defined levitation area. Similar to the use of a standard hemocytometer, the cells within a set region (i.e., the FOV of one image) are counted; this process is repeated in 10 randomly selected regions throughout the capillary tube by shifting the sample through the magnets slightly, capturing an image at each location. The WBCs, defined as particles within the levitation region set in Error! Reference source not found.a, may be counted in each image and averaged. The width of the region in the FOV is 1 mm and the cross-sectional area of the capillary is 0.7 mm by 0.7 mm. Thus, the volume of the sample in each FOV is approximately 0.5 μL. Multiplying the average number of WBCs per FOV by a factor of 2000 will correct for this volume and the sample dilution (1:1000 in this experiment) to give the number of cells per microliter (where the normal range is generally considered to be 3500–11000 WBCs/μL).

Where other technologies use lysis buffers to remove RBCs or stains to identify the WBC population, this approach can perform density-based separation of RBCs and WBCs and isolate the WBC population with no lysis step necessary. Error! Reference source not found.e shows the effective separation of the RBC and WBC populations in 25 mM Gd after 10 min. The RBCs levitate at a lower height (with many along the bottom of the capillary) than the WBCs. This result is consistent with previous literature: the majority of the WBC population has been reported to be less dense than the RBC population (as calculated via...
Figure 29 Label-free identification of WBCs in blood

(a) Confinement of WBCs without RBCs (25 mM Gd, 10 min levitation period); the WBC confinement region is outlined in blue. (b) Schematic for WBC quantification: the sample is moved through the space between the magnets to capture 10 images randomly spaced throughout the entire sample contained in the capillary tube. Each imaged region is about 1 mm in width and represents a 0.5 μL volume. The total concentration of WBCs per microliter in the sample is equal to the average number of WBCs per FOV multiplied by a factor of 2000. (c) Separation of WBCs (blue arrows) from RBCs (below).

Reproduced, with permission, from (Yenilmez, et al., Adv Mat Tech, 2016b)
density gradient centrifugation[33]). Further, different types of WBCs have differing densities ranging from 1.060 to 1.110 g/cc[33], which explains the broad spread of levitation heights observed. A concentration of 25 mM Gd was selected for this application in order to achieve the maximum possible separation between the RBC and WBC populations (for more reliable detection) without allowing the WBC population to reach the bottom of the range allowed by the sample tube.

**Comparison with existing technologies**

In contrast to other automated devices (compared in Table 5), the simplified process presented here does not require RBC lysis reagents or stains to identify WBCs. Rather, this test leverages the unique inherent densities of WBCs compared to RBCs to perform rapid, label-free separation. For this reason, the cost of the consumables is less than $1 per test, comprising only a sample tube and a low-cost paramagnetic medium. While additional clinical studies must be performed to validate this application in a large patient population, these results demonstrate promise for WBC cytometry as well as other applications to quantify a particular population of cells in blood or in other body fluids such as urine or saliva.

| Required equipment | Self-contained magnetic levitation platform | Microscopy | Flow cytometry  
(Sysmex XE 2100)[14] | Hemocue WBC System[15] |
<table>
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<tr>
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<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment cost</td>
<td>Compact, handheld device</td>
<td>Microscope</td>
<td>Flow cytometer</td>
<td>Hemocue system</td>
</tr>
<tr>
<td>$&lt;$ 100</td>
<td>$&gt;$ 500</td>
<td>$15,000</td>
<td>$600</td>
<td></td>
</tr>
<tr>
<td>Cost per test</td>
<td>$&lt;$ 1</td>
<td>-</td>
<td>$10 (stains)</td>
<td>$1 (cuvette)</td>
</tr>
<tr>
<td>(microcapillary, Gd, SMBS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doctor/technician skill</td>
<td>Simple interface, straightforward sample loading, no knowledge necessary</td>
<td>Procedure is tedious and requires trained technician</td>
<td>Procedure is tedious and requires trained technician</td>
<td>Simple interface, straightforward sample loading</td>
</tr>
<tr>
<td>Time</td>
<td>10-15 min</td>
<td>10-15 min</td>
<td>$&gt;$ 60 min</td>
<td>5 min</td>
</tr>
</tbody>
</table>
Section 6.3: Other potential applications

In conversations with clinicians, one of the diagnostics that was notably lacking in POC diagnosis options, and was most highly desired among the clinicians who were interviewed, was bacterial infections. Hence, we tested the ability to levitate and image bacterial cells in the proposed system. This is an optical imaging challenge, as bacterial cells are very small (for example, *Escherichia coli* are rod-shaped with radius 0.5 μm and length around 2μm); hence, individual cells cannot be resolved with the present imaging system. Therefore, an improved imaging system was developed by replacing the stock lens included with the Raspberry Pi camera with a 20X objective (BD Plan 20/0.4, Nikon) with numerical aperture of 0.4. A microscope objective is substituted for the stock lens included with the Raspberry Pi camera. The objective is a 20X objective (BD Plan 20/0.4, Nikon) with numerical aperture of 0.4. The system is shown in Figure 30. To optimally focus the system, the lens equation shown in Equation 15 was utilized given that \( u \) was the distance T2L and \( v \) was the distance L2C in Figure 30:

\[
\frac{1}{f} = \frac{1}{u} + \frac{1}{v} = \frac{1}{T2L} + \frac{1}{L2C}
\]  

Solving Equation 15 for \( f \), and optimizing \( v \) for several values of \( u \) by moving the camera until the image was in focus, \( f \) was determined to be about 3.6 for this lens.

The imaging capabilities of the existing optical setup in the self-contained device described above are shown in Figure 31 (top) and those obtained with the modified optical system are shown in Figure 31 (bottom). These results show that single bacteria can be resolved much better using the proposed optical
The dimension of each bacterial cell is around 8 pixels, which is sufficient to perform automated particle detection. However, a challenge noted with imaging of bacterial cells is that, while most were confined over the course of several hours, a subpopulation tended to be motile. This may be due to random Brownian motion of the very small particles or self-propulsion via the flagella of this species of bacteria, which overcomes the magnetic forces present in the field. A video demonstrating this phenomenon can be seen here: https://youtu.be/RW6Khx1VVP0. Further, the cells can be seen moving in the focal plane as well as coming in and out of focus (i.e. in and out of the focal plane moving along the imaging axis).

Figure 31 E. coli imaging with the current optical system and adapted optical system (top) Imaging with the existing optical system in the self-contained setup shown above and (bottom) imaging with the adapted optical system shown in Figure 30.
Further studies are required to increase the depth of field to capture more of each bacterial traveling path as well as to characterize the motion of the bacteria. To our knowledge, such results regarding bacterial motility in a magnetic field have not been reported previously. However, this serves only as preliminary data and further work should be done to characterize the imaging capabilities and validate these findings in clinical samples.
Chapter 7: Aim 4: Investigation of the commercial potential

The final goal was to investigate the commercial potential of this concept by speaking directly with key players in the POC diagnostics market. The ultimate goal of engineering research is to positively impact the world. In order for technology developed in academic research to have an impact on society, it is critical to consider the translation of the technology into a commercial product. Thus, we will conduct a process of customer discovery according to the NSF I-Corps Framework[68] through the Accelerate UConn program. This program is designed to accelerate the translation of basic-research projects into commercial products. It is a hypothesis-driven program that emphasizes interactions with important players in the market at all levels to validate our assumptions and iterate on the business model. Specifically, regarding potential customer segments and the value propositions to those customer segments, we aim to validate two hypotheses: (1) remote testing causes delays in deciding the proper course of treatment and (2) clinicians are willing to add POC testing to their workflow in order to gain results on-site. Moreover, regarding customer relationships and distribution channels, we aim to validate several other hypotheses: (1) healthcare providers will respond to a sales team and buy devices directly from a startup company, (2) a technology support team and data monitoring team will be necessary, and (3) insurance billing/coding partnerships will be required for successful implementation.

Section 7.1: Clinical considerations

The first major finding was regarding the value of POC diagnostic tools. In most medical offices that were visited, there is at least some infrastructure already in place to carry out POC testing. However, this infrastructure varied in terms of capabilities from a small bench in the office (a small private practice) where simple tests such as dipsticks and pregnancy tests are run to entire floors where complex testing is carried out. In some cases, there is dedicated diagnostic testing support staff but in others, the nurses are generally responsible to carry out these tasks. In all interviews with clinicians (not only doctors but nurses and support staff, as well), there was a strong consensus that the clinicians’ time is extremely limited. Thus, process delays are not only inconvenient but also expensive in terms of the practice’s bottom line.
The cost of labor-intensive processes was most strongly noted in the privately owned practices. Thus, there was a strong preference for diagnostic tools that are not labor-intensive. Most tests that are currently incorporated in the workflow only take a few minutes of the clinician’s time – those that take longer would not be favored and would likely instead be sent out. Wait times of up to 15 minutes are generally considered acceptable as the clinician can leave the and return for the results as needed. However, longer wait times would mean that the patient would be taking up a bed while waiting for the results, which affects the throughput of the office. Therefore, it is important to limit the hands-on time as well as the overall test time. The current prototype requires only about 2 minutes of sample preparation and 10 minutes of waiting time, which should be within the established thresholds.

In all practices, particularly those with limited diagnostic capabilities, there is an established infrastructure for sending out tests that are too complex to conduct given the resources of the practice or that require expensive equipment that is not available at that site. These outsourced tests are commonly encountered but seem to be pain point in some specific cases. For example, the hemoglobin electrophoresis testing used to monitor SCD patients generally takes a few days, meaning that a doctor does not have enough information to treat the patient at regular checkups. This means that the patient is required to the diagnostics laboratory beforehand, which can be inconvenient and costly. Further, if the patient forgets to do the proper testing, the doctor can only provide limited care in the scheduled appointment period with the information needed to treat the patient. Similarly, when diagnosing infections, it is critical to obtain the results in a timely manner in order to prescribe treatment accordingly before the infection spreads and becomes even more difficult to treat. In cases where there is a severe risk associated with delaying treatment, the physician will often take an “educated guess” regarding the appropriate treatment based on symptoms and prior experience. Moreover, tests to determining the antibiotic resistances and susceptibility are also important for prescribing the correct antibiotics. For example, a UTI can sometimes be diagnosed using a urine dipstick. However, further testing is needed to determine which antibiotics the particular strain is resistant to; this is generally done by swiping the
sample onto an agar plate that is preloaded with different antibiotics in specific regions, incubating the plate for two to three days, then observing the colony growth in each of the regions. Yet, in this period of two to three days, there is a risk that the urinary tract infection may spread to the bladder and even the kidneys, which is far more dangerous and difficult to treat. Thus, a physician will often make an educated guess in prescribing an antibiotic based on known antibiotic resistances of existing bacterial strains in the region. However, in many cases, this guess is incorrect (one clinician reported about 1 in 5 cases), meaning that the patient would need to return to the clinic and be prescribed a different antibiotic once the results are available, risking worse infection and wasting time and money for all players involved. Thus, it would be extremely useful to have a rapid and portable tool to conduct the same measures while a patient is in the office or even at home for applications such as these.

Returning to the point that a doctor’s time is money, the inefficient process of sending samples out for testing, waiting for the results, logging the results in the patient record, and calling a patient or holding another appointment are all pain points in medical practices. While some tasks are reimbursable, may come from the office overhead and, thus, reduce the profitability of the practice. Therefore, having these testing capabilities on-site would be highly desirable, and notably worth the fixed cost of purchasing a platform device, particularly for commonly performed tests. Another useful feature to reduce the work required by the clinician would be automated integration with patients’ electronic health records systems (which were used in almost every office that was studied here.)

Another important insight gained by speaking directly with the clinicians who carry out the POC diagnostic testing is the need for visible quality control measures. In one practice, each of the several clinicians that were interviewed reported generalized distrust of their instrument to read urine dipsticks. The urine dipstick is an array of chemical assays that is dipped in a cup of urine and changes colors according to the urine content. Generally, the colors can be read by eye but this practice has purchased POC instruments to automatically analyze the colors and return quantitative results. However, several nurses reported that the instrument often gave very unexpected readings. In such cases, they would have
to run the test again and read it by eye, which was a waste of time. However, this represents a larger problem, as there is no way to tell if the readings are correct or may be in error for any particular patient – there were no visible quality controls that could be run with every sample to ensure proper functioning of the instrument. On the particular day of the interviews, they had received several suspect readings and had stopped using the instrument altogether. Thus, it is critical for the success of new POC technologies such as that proposed here that there are visible quality control measures, including those that can be run at any time the user feels that the results may be suspect.

Finally, internal medicine clinicians were asked which tests they most commonly conduct and which would be most beneficial for use on-site. Overwhelmingly, tests for sexually transmitted diseases and urinary tract infections were the most commonly requested. Thus, these areas are considered to be the most promising directions for future development of new applications of the proposed platform technology.

Section 7.2: Regulatory considerations

POC diagnostic devices are regulated under the Food and Drug Administration by the Clinical Laboratory Improvement Amendment (CLIA). There are certain classifications for diagnostic devices: waived, moderate complexity, and high complexity. Waived tests can be obtained over the counter and performed at home or in a practice with minimal regulation; they include urine dipsticks, standard fingerstick glucose testing, and pregnancy tests. On the other hand, moderate- and high-complexity tests are more difficult to carry out or pose a higher risk and, thus, are subject to stronger restrictions on which practices can carry them out. Under CLIA, laboratories that conduct moderate- or high-complexity tests are subject to a set of laboratory standards regarding certification, personnel, proficiency testing, patient test management, quality assurance, quality control, and inspections. Three federal agencies are responsible for CLIA: The Food and Drug Administration (FDA), in additional to approving new tests, categorizes them into these three categories based on their complexity; it also reviews and approves requests for Waiver by Application for new tests and publishes guidance documents regarding the regulations. The
Center for Medicaid Services (CMS) conducts inspections, enforces compliance, and certifies laboratories to carry out the different categories of tests; it monitors laboratory performance via Proficiency Testing (PT). Further, the Center for Disease Control (CDC) provides analysis, research, and technical assistance in the development of technical standards and laboratory practice guidelines and monitors the PT practices.

Representatives from these agencies or clinicians that are directly experienced with their activities were interviewed. Many practices, particularly smaller ones, notably were only able to conduct waived testing due to the burden of the PT program and the high fees associated with certification to conduct moderate-and high-complexity tests. While some were certified to conduct moderate-complexity tests, even fewer to able to conduct high-complexity tests on site due to space, personnel, and financial limitations.

For this project, we anticipate that the tests could be FDA approved with sufficient clinical data via the 510(k) approval process based on the fact that there are existing predicate devices for all the proposed tests. It would be necessary to prove that the proposed device is “as good as” existing predicate technologies with sufficient statistical power. Further, it will be important to identify the criteria considered for CLIA classification and aim to meet the criteria for being a moderate-complexity test (and perhaps even a waived test in future iterations). Each test is graded for level of complexity based on the sum of the scores (1, 2, or 3, where 1 indicates the lowest level of complexity and 3 represents the highest complexity) for each of the seven criteria on the scorecard[69]: knowledge; training and experience; reagents and materials preparation; characteristics of operational steps, calibration, quality control, and proficiency testing materials; test system troubleshooting and equipment maintenance; and interpretation and judgment. A total score of 12 or less indicates that the test can be categorized as moderate complexity while a score above 12 indicates that it must be categorized as high complexity. These design criteria were considered throughout the design process and can be integrated into future product development.
Insurance agencies greatly influence technology adoption as they decide which procedures they will reimburse the physician for. Further, insurers have some control over a physician’s practices because, if a practice wishes to remain “in network” with a particular insurer, they must comply with certain policies and procedures, which may include diagnostic protocols, that are set by the insurer.

In terms of pricing, the reimbursement for procedures is often done at a rate that has been previously negotiated between the insurer and the healthcare provider. Only recently has CMS required hospitals to publish online a list of their standard charges in a machine-readable format and update the information at least once per year. However, these negotiated “fee schedules” are not widely publicly available, so it is difficult to determine the target price per test to be comparable with the current reimbursement rates.

Regarding the coverage of new tests by insurers, anecdotal evidence suggests that an insurance company would be willing to cover a new test if it offers value to the patient (i.e., reduced wait time) or healthcare provider (i.e., reduce burden on the clinicians) but only if the benefit is commensurate with the cost compared to the next alternative. There is an entire field or research, referred to as Health Economics and Outcomes Research (HEOR) that is utilized to make such decisions. However, the insurance company requires a significant amount of data to make evidence-based coverage decisions: data about the analytical and clinical validity of a test as well as its clinical utility is needed, though not always readily available. Further, this information should be specific to the populations served by the health insurance plan (for example, Medicare requires data on individuals age 65 and older). A cost-effectiveness analysis is conducted based on the costs and health outcomes of an intervention relative as compared to the alternative (e.g., using an alternate technique or not conducting the test at all). This is sometimes measured as the difference in cost per quality-adjusted life-year (QALY) gained.

Further investigation of this topic would likely require direct collaboration with a healthcare insurer and extensive research to evaluate the economics of the proposed test.
Chapter 8: Conclusions

Current diagnostic technologies are either insufficient for point-of-care applications, requiring expensive peripheral equipment or specialized training, or in the case of paper tests, provide only qualitative results which must be interpreted by a trained user, leaving room for human error (Table 4 and Table 5). The approach proposed here is low cost in terms of the diagnosis platform (<$100) and the cost per test (<$1). The assay requires only a drop of blood obtained via finger stick and sample preparation involves mixing with a prepared solution, loading into a microcapillary via capillary action, and simple user interface with the platform. The platform then automatically analyzes the samples and provides quantitative and reliable results in under 15 min. This is a viable approach to provide ubiquitous and accessible medical diagnostics, preventing serious complications which can arise from the disease going undiagnosed in newborns in low-resource settings and developing countries[70].

The main intellectual merit of this thesis is that, unlike previous magnetic levitation-based technologies, the developed platform is completely automated and fully independent from a dedicated microscope or a smartphone, increasing the portability and accessibility of this tool particularly for field applications (Table 6). This involved the integration of three main components:

1. magnetophoresis (the use of magnets to manipulate single cells based on their intrinsic properties);

2. micro-scale imaging (optimization of optical systems to image the levitating cells, which are around 8–12 µm in diameter, with some being optically transparent); and

3. image processing (analyzing the captured digital images to evaluate the distribution of cells within the magnetic field and interpret the distribution as an indicator of disease)

The development of this system, and the extensions thereof, was enabled by the use of CAD for rapidly iterating on designs and 3D printing for rapid prototyping.
Table 6 Technologies for label-free single-cell manipulation using magnetophoresis

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<tr>
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<th>Whitesides group9-17</th>
<th>Demirci group[38]</th>
<th>Proposed platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnet size</td>
<td>5 by 5 by 2.5 cm</td>
<td>2 by 5 by 50 mm</td>
<td>2 by 5 by 50 mm</td>
</tr>
<tr>
<td>Particle size</td>
<td>Must be visible to the naked eye</td>
<td>Micro-scale (few to hundreds µm)</td>
<td>Micro-scale (few to hundreds µm)</td>
</tr>
<tr>
<td>Required equipment</td>
<td>Magnet setup with a holder for the sample container, camera, ruler</td>
<td>Magnet setup, microcapillary tube, microscope flipped on its side</td>
<td>Self-contained or smartphone-based setup, microcapillary tube</td>
</tr>
<tr>
<td>User input</td>
<td>Measurements by hand</td>
<td>Imaging done by microscope, image analysis done by hand</td>
<td>Fully automated</td>
</tr>
<tr>
<td>Complexity</td>
<td>Difficult and prone to user error</td>
<td>Medium, requires skill to operate microscope</td>
<td>Low, could be performed by an untrained user</td>
</tr>
</tbody>
</table>

The broader impacts of this work include the development of a widely applicable and accessible diagnostic tool. Existing state-of-the-art clinical diagnostic tools are highly complex and costly and, thus, not compatible with on-site measurements. Hence, samples must be transported to a remote clinical testing laboratory for testing. This process can cause delays in diagnosis and, therefore, delays in administering the proper treatment; this is not only inconvenient but also poses a risk that the patient’s condition will become worse during the time it takes to conduct the prescribed diagnostic testing.

Existing on-site testing technologies only partially mitigate the issues; however, they only provide limited data, often suffer from lower sensitivity and specificity compared to the standardized clinical tests and are generally higher in cost per test than sending the sample to a lab for testing. Moreover, many important diagnostic tests are not available as on-site tests. Therefore, the proposed platform, which is inexpensive in terms of equipment and per-test costs, rapid, and automated and does not require extensive sample preparation, labeling, or user skill, is expected to facilitate the practical implementation of a wide variety of medical diagnostics at the POC, in limited-resource settings, and at home.

Future work should focus on further clinical validation of the proposed diagnostic tests using larger sample sizes. For SCD diagnosis, it will be important to test samples from patients who have yet to be treated for the condition to ensure that the test is applicable to this population, as well. Further, testing
over a larger sample size will allow us to set a better threshold to distinguish between healthy and SCD-positive. For broad screening application, it may be best to select a low threshold to ensure that all subjects who have SCD are captured. Furthermore, additional work is needed to validate the possibility of conducting SCD monitoring. While the expected trends were observed, there was still significant variability in each of the distribution metrics tested. Thus, further work to identify a better metric based on a larger dataset is required to claim the ability to accurately monitor SCD status over time. For the WBC cytometry application, we demonstrated a proof of concept with a single patient. The test should be repeated on many subjects and the results should be compared with those obtained by clinically accepted methods to evaluate the accuracy and precision of this test. Moreover, additional applications based on the density and size differences of cells and another diamagnetic particles may be investigated[71].
Chapter 9: Summary of Publications

Below is a summary of the publications resulting from my doctoral research work. I have contributed to 25 articles. Many articles were products of the research described herein but I have also contributed to several other projects.

Section 9.1: Magnetophoresis for global health

The following articles are related to the topic of this thesis: in vitro diagnostic tools based on magnetophoresis. Some of the content above was reproduced, with permission, from these published articles as described below.

Research Articles

1) Smart-Phone Based Magnetic Levitation for Measuring Densities (2015)
   S Knowlton, CH Yu, N Jain, IC Ghiran, S Tasoglu
   *PLOS ONE*, 10 (8), e0134400
   *This was the foundational work on this topic describing the adaptation of magnetophoresis technology for POC applications in a smartphone-based setup.*

2) Smart-phone attachable, flow-assisted magnetic focusing device (2016)
   R Amin, S Knowlton, B Yenilmez, A Hart, A Joshi, S Tasoglu
   *RSC Advances*, 6 (96), 93922-31
   *This was an adaptation of the smartphone-based device to conduct cell separation in a continuously pumped sample.*

3) 3D-Printed Smartphone-Compatible Point of Care Tool for Fluorescence- and Magnetophoresis-Based Cytometry (2017)
   S Knowlton, A Joshi, P Syrrist, S Tasoglu
   *Lab on a Chip*, 17, 2839-2851
   *This work represents the incorporation of fluorescence-based imaging capabilities to extend the applicability of the platform device.*

4) Sickle Cell Detection Using a Smart Phone (2015)
   S Knowlton, I Sencan, …, MM Heeney, IC Ghiran, S Tasoglu
   *Scientific Reports*, 5, 15022
   *In an extension of the original smartphone-based work, we further demonstrated the ability to distinguish sickle cells from healthy cells in the setup.*

5) Label-free Sickle Cell Disease Diagnosis Using a Low-Cost, Handheld Platform (2016)
   B Yenilmez*, S Knowlton*, M Heeney, S Tasoglu (*co-first authors)
   *Advanced Materials Technologies*, 1(5)
   *Here, we demonstrated the use of the self-contained device for SCD diagnostics.*

6) Self-Contained Handheld Magnetic Platform for Point of Care Diagnostics (2016)
This represents the transformation of the smartphone-based device developed previously to a self-contained version. This includes an extension to WBC cytometry.

7) 3D-printed Smartphone-based Device for Label-free Cell Separation (2017)
   R Amin, S Knowlton, …, JJ Zhao, S Tasoglu
   Journal of 3D Printing in Medicine 1(3)
   This article demonstrates the separation of cancer cells. I contributed to the culture and testing of the four cancer cell lines.

Video Articles

8) Magnetic Levitation Coupled with Portable Imaging and Analysis for Disease Diagnostics (2016)
   S Knowlton, B Yenilmez, R Amin, S Tasoglu.
   JOVE, e55012-e55012
   This was a summary of the above articles in a different form of scientific communication, which is important in light of the growing role of digital media in disseminating research findings.

Section 9.2: Tissue engineering

Research articles

9) 3D-Printed Microfluidic Chips with Patterned, Cell-Laden Hydrogel Constructs (2016)
   S Knowlton, CH Yu, F Ersoy, S Emadi, A Khademhosseini, S Tasoglu
   Biofabrication, 8(2), 025019

10) Development and characterization of a low-cost 3D bioprinter
    B Yenilmez, M Temirel, S Knowlton, E Lepowsky, S Tasoglu
    Bioprinting, In press

Invited Book Chapters

11) Chapter 4: Building Blocks for Bottom-Up Neural Tissue Engineering: Tools for In Vitro Assembly and Interrogation of Neural Circuits (2016)
    S Knowlton, D Li, F Ersoy, Y Cho, S Tasoglu
    Neural Engineering, Springer International Publishing, pp. 123-144

Invited Review Articles

12) Bioprinting for cancer research (2015)
    S Knowlton, S Onal, CH Yu, JJ Zhao, S Tasoglu
    Trends in Biotechnology, 33(9), 504-513

Invited Short Opinion Articles

13) 3D Printed Body on a Chip for Military Applications (2017)
R Amin, S Knowlton, B Yenilmez, S Tasoglu
*HDIAC Journal*, 4(3), 27-29

   S Knowlton and S Tasoglu
   *Trends in Biotechnology*, 34(9), 685-688

15) A bioprinted liver-on-a-chip for drug screening applications (2016)
   S Knowlton and S Tasoglu
   *Trends in Biotechnology*, 34(9), 681-682

**Review Articles**

16) Utilizing stem cells for three-dimensional neural tissue engineering (2016)
   S Knowlton, Y Cho, XJ Li, A Khademhosseini, S Tasoglu
   *Biomaterials Science*, 4(5), 768-784

17) Bioprinting for Neural Tissue Engineering (2017)
   S Knowlton, S Anand, T Shah, S Tasoglu
   *Trends in Neurosciences*, 41(1), 31-46

18) Photocrosslinking-Based Bioprinting: Examining Crosslinking Schemes (2017)
   S Knowlton, B Yenilmez, S Anand, S Tasoglu
   *Bioprinting*, 5, 10-18

19) 3D-Printed Microfluidic Devices (2016)
   R Amin, S Knowlton, A Hart,.. A Khademhosseini, S Tasoglu
   *Biofabrication*, 8(2), 022001

20) Advancing Cancer Research Using Bioprinting for Tumor-on-a-Chip Platforms (2016)
   S Knowlton, A Joshi, B Yenilmez, IT Ozbolat, CK Chua, A Khademhosseini, S Tasoglu

**Section 9.3: Other topics of research**

**Research articles**

   R Amin, F Ghaderinezhad, L Li, E Lepowsky, B Yenilmez, S Knowlton, S Tasoglu
   *Analytical Chemistry*, 89(12), 6351–6357

22) Three-Dimensional-Printed Carnivorous Plant with Snap Trap (2016)
   M Temirel, B Yenilmez, S Knowlton, J Walker, A Joshi, S Tasoglu
   *3D Printing and Additive Manufacturing*, 3(4), 244-251

**Review Articles**

23) Microfluidics for sperm research (2015) *invited review
   S Knowlton, M Sadasivam, S Tasoglu
   *Trends in Biotechnology*, 33(4), 221-229

S Tasoglu, HC Tekin, F Inci, S Knowlton, …, U Demirci
Proceedings of the IEEE, 103(2), 161-178

E Lepowsky, F Ghaderinezhad, S Knowlton, S Tasoglu
Biomicrofluidics, 11(5), 051501
Chapter 10: Works Cited


