High-Throughput Density Measurement Using Magnetic Levitation

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S Supporting Information

ABSTRACT: This work describes the development of an integrated analytical system that enables high-throughput density measurements of diamagnetic particles (including cells) using magnetic levitation (MagLev), 96-well plates, and a flatbed scanner. MagLev is a simple and useful technique with which to carry out density-based analysis and separation of a broad range of diamagnetic materials with different physical forms (e.g., liquids, solids, gels, pastes, gums, etc.); one major limitation, however, is the capacity to perform high-throughput density measurements. This work addresses this limitation by (i) re-engineering the shape of the magnetic fields so that the MagLev system has a capacity with 96-well plates, and (ii) integrating a flatbed scanner (and simple optical components) to carry out imaging of the samples that levitate in the system. The resulting system is compatible with both biological samples (human erythrocytes) and nonbiological samples (simple liquids and solids, such as 3-chlorotoluene, cholesterol crystals, glass beads, copper powder, and polymer beads). The high-throughput capacity of this integrated MagLev system will enable new applications in chemistry (e.g., analysis and separation of materials) and biochemistry (e.g., cellular responses under environmental stresses) in a simple and label-free format on the basis of a universal property of all matter, i.e., density.

INTRODUCTION

Density is a fundamental physical property of all matter: the density of homogeneous matter (solids, liquids, gels, or gases) is simply described by the ratio of mass to volume \( \rho = \frac{m}{V} \). Heterogeneous matter (composites, polymers with amorphous and crystalline regions, or phase-separated regions) has a density averaged over the volumes of the different subregions described by the same formula, since these regional densities may differ. Changes in density are associated with changes in both physical and chemical properties of a material. (For example, the densities of polymers, such as solid plastic parts, may depend on their method of fabrication, or following chemical degradation when they are exposed to UV irradiation or acids/bases.) Cells of different types have different densities. (For example, the densities of erythrocytes are different than the densities of adipocytes rich in fat, and even than sickled erythrocytes.)

Density is broadly useful to separate, characterize, and/or identify both biological and nonbiological materials. Density-based methods have been used routinely to characterize materials, to separate, isolate, or fractionate subpopulations from complex mixtures, and to follow changes in density in a wide range of systems (e.g., responses of biological cells to drug treatments, such as bacteria, and chemical reactions, such as polymerization).

Existing analytical methodologies, from simple centrifugation-based methods (e.g., Percoll gradient centrifugation) to more specialized techniques and types of instrumentation (e.g., methods based on pycnometers, density gradient columns, or vibrating tube densitometers), are important routine methods. More complex approaches (e.g., microfluidics-based approaches using cantilever-based microresonators) are also expanding the uses of density.

We also recently developed two distinct but complementary approaches to perform density measurements and/or separations: (i) One is based on the use of aqueous multiphase polymer systems (AMPS), mixtures of polymers that form distinct phases with different densities in water. These phases are separated by interfaces that are sharp on the molecular scale; these phases and interfaces can be used to perform density-based separations. (ii) The second is magnetic levitation (MagLev), a technique that uses competing gravitational (buoyant) and magnetic forces to form an effectively

Received: February 1, 2018
Published: June 11, 2018

DOI: 10.1021/jacs.8b01283
J. Am. Chem. Soc. 2018, 140, 7510−7518
continuous density gradient in an aqueous paramagnetic medium in a magnetic field, and allows separations of particles suspended in the medium on the basis of their densities.\(^9\) MagLev using permanent magnets and paramagnetic media, as we have been developing it, is a versatile tool for measurement of density.\(^9\) It is broadly applicable to a wide variety of samples.\(^9\)

The device most commonly used for MagLev (Figure 1) consists of two NdFeB permanent magnets placed coaxially with like-poles facing, and uses a cuvette filled with a paramagnetic medium as the container to levitate samples (when placed between the two magnets).\(^10,11\) (MagLev is distinct from the magnetic separation technologies common in biochemistry, which use superparamagnetic particles and involve magnetic fields to separate these particles from diamagnetic suspensions.\(^12–14\)) The central axis of the MagLev device is aligned with the gravitational field, and the objects levitate vertically along this axis. In this configuration, MagLev covers a density range of \(-0.8 \text{ to } 3 \text{ g/cm}^3\) when the paramagnetic solution is a concentrated solution of common paramagnetic salts such as MnCl\(_2\) and GdCl\(_3\).\(^9\) Depending on the dimensions and orientation of the magnets, the metrics for analytical performance (primarily dynamic range and sensitivity) can be appropriately optimized to cover the entire range of these gradients in space so that the device is compatible with one of the most common types of containers for arrays of small (up to \(\sim 300 \mu\text{L}\)) liquid samples in the laboratory, a 96-well plate.

This format used long and thin magnets (\(L \times W \times H\) for each indistinguishable magnet: 101.6 mm \(\times\) 4.8 mm \(\times\) 6.4 mm). These magnets were inserted in the spaces between the rows of tubes on a 96-well plate with the like-poles facing one another. This format allows parallel reading using indistinguishable (in principle) magnetic fields around each well.

Finite-element simulation in COMSOL evaluated the profile of the magnetic field in the gaps of the magnet array. The dimensions used for the array generated both a strong magnetic field (up to \(\sim 0.7 \text{ T}\) along the central z-axis in the gaps) and field gradient, and, therefore, required low concentrations of paramagnetic salts to levitate samples (e.g., \(\sim 0.5 \text{ M MnCl}_2\), covers a range of densities from \(\sim 1.0 \text{ g/cm}^3\) to \(\sim 1.6 \text{ g/cm}^3\), which would otherwise require \(\sim 3 \text{ M MnCl}_2\) to cover the same range in the “standard” MagLev device\(^9\)). Low concentrations of paramagnetic salts improve the biocompatibility of the suspending media, and are particularly useful for levitating biological cells.

Parallel density measurements across the entire 96-well plate validated the performance of the system—and particularly its reproducibility. We demonstrated broad applicability of high-
throughput density measurements of biological and non-biological materials in aqueous paramagnetic media using this MagLev-based system for three representative types of samples: (i) surfactant-stabilized drops of 3-chlorotoluene (a hydrophobic liquid), (ii) small solid particles and powders (crystals of cholesterol, glass particles, copper powder, and polymer beads), and (iii) human erythrocytes.

MagLev has eight important characteristics that make it useful in performing high-throughput density measurements: (i) MagLev is simple in design and use. It uses permanent magnets to generate a magnetic field, and does not require electricity to operate (though the scanner we used to image the device requires electrical power). The device can be assembled simply, and be used, in principle, indefinitely without maintenance beyond occasional calibration. (ii) MagLev usually uses inexpensive, commercially available paramagnetic salts (especially MnCl₂) to generate appropriate media in aqueous solutions. (DyCl₃ is also sometimes particularly useful for its high magnetic susceptibility.¹¹) (iii) MagLev is a label-free method that directly measures the densities of the materials. It does not require chemical derivatization or labeling (e.g., with fluorophores). (iv) It can be used in a format that enables parallel measurements and, thus, the ability to do high-throughput measurements of density (this paper). (v) MagLev as we describe here covers a wide range of densities, from an air bubble (~0 g/cm³), using 3 M DyCl₃, data not shown) to copper particles (8.96 g/cm³), and has a tunable sensitivity in density measurements (this study demonstrated Δρ as small as ~0.001 g/cm³ using 0.1 M MnCl₂). (vi) MagLev requires only small quantities of samples. (It can easily detect single colored particles ~200 μm in diameter.) It can be applied to a variety of samples with different physical forms (e.g., heterogeneous, sticky, fragile, and/or irregularly-shaped samples). (vii) MagLev enables measurements over a convenient interval of time (typically a few minutes to <1 h for a single scan of the plate). (viii) The configuration of MagLev we describe here is compatible with a simple flatbed scanner for imaging purposes.

The major limitations of the system we describe include (i) inability to measure densities of samples having a large size (e.g., mm or above), and (ii) requirement of paramagnetic media that do not dissolve (or react with) the materials of the plates or the sample. (This study used plates made of polypropylene.)

The ability of high-throughput density measurements using MagLev will, as in other areas of analytical chemistry and biochemistry, generate new applications. In particular, the simplicity and affordability of the system expands the range of applications for which it might be used.

### EXPERIMENTAL DESIGN

#### Theory of MagLev

Previous papers have described the theory of MagLev⁶,¹¹ and the description below includes only an overview of the “standard” MagLev device and the key equations used to guide the design of the current, “parallel” MagLev device.

The “standard” MagLev device (Figure 1) comprises two NdFeB permanent magnets positioned with the like-poles facing (L × W × H for each indistinguishable magnet: 50.8 mm × 50.8 mm × 25.4 mm) and coaxially at a distance of 45.0 mm to yield an approximately linear magnetic field along the central axis between the magnets. This central axis aligns with the vector of gravity. When a diamagnetic sample is placed in a container filled with a paramagnetic medium and then centered coaxially in the device, the sample can float, or levitate, stably without physically contacting the container. At equilibrium, the gravitational force Fg acting on the object (corrected for the effect of buoyancy) balances the magnetic force F mag which the sample experiences as a result of the direct interaction of the magnetic field and the paramagnetic medium that surrounds the sample. The levitation height h, the distance from the centroid (the geometric center) of the sample to the top surface of the bottom magnet, is proportional to the density of the sample, and therefore, can be measured experimentally (with appropriate calibrations using known density standards) to calculate the unknown density of the sample.

Equation 1 describes the balance of physical forces acting on the levitated sample. Equation 2 shows that the density of the sample that levitates at a given position can be calculated (to a good approximation) using the characteristics of the suspending medium (including its density, ρ mag and magnetic susceptibility, χ m) the magnetic susceptibility of the sample, χ s (which is usually negligible in comparison to χ m), and the strength and gradient of the magnetic field B, (dB/dz) at the position of equilibrium.

\[
\vec{F}_g + \vec{F}_{mag} = (\rho - \rho_m)\vec{g} + \frac{(\chi_s - \chi_m)}{\rho_s} V(\vec{B} \cdot \vec{V})\vec{B} = 0
\]

\[
\rho_s \approx \frac{\chi_s - \chi_m}{\mu_0 g} \left( B_0 \frac{dB}{dz} \right) + \rho_m
\]

In eqs 1 and 2, F g (N) is the gravitational force corrected for the effect of buoyancy, F mag (N) is the magnetic force, ρ s (g/cm³) is the density of the suspended sample, ρ mag (g/cm³) is the density of the paramagnetic medium, g (9.810 m/s²) is the vector of gravity, χ m (unitless) is the magnetic susceptibility of the paramagnetic medium,
χ (unless)” is the magnetic susceptibility of the suspended sample, \( \mu \) (4π \( \times 10^{-7} \) N/A²) is the magnetic permeability of free space, \( V \) (m³) is the volume of the object, \( B (T) \) is the magnetic field, \( V \) is the gradient operator, \( B_z \) is the z-component of the magnetic field, and \( (\partial B_z / \partial z) \) is the gradient of the z-component of the field along the central z-axis.

This work exploited the magnetic field gradient that is perpendicular to the central z-axis of the “standard” configuration, and aligned it to the gravity, to levitate samples suspended in paramagnetic media (Figure 2A). To illustrate this design, we rotated the “standard” configuration 90° in the x-z plane about the geometric center. The field gradient exploited in the “standard” configuration now became horizontal (the solid white line, Figure 2A); the field gradient exploited in this study (highlighted by the solid red line, Figure 2A) became aligned with that of gravity. (The force of gravity is constant across the sample.) The linear range of this gradient (bounded by the open red circles, Figure 2A) is functionally similar to the gradient along the central axis in the “standard” configuration for levitating samples in paramagnetic media (against gravity). When a sample is suspended in a paramagnetic medium and then placed in this magnetic field, the sample will experience a magnetic force that pushes it toward the central z-axis, and simultaneously reach its position of equilibrium along the z-axis based on the balance of physical forces along this axis (eq 1). Equations 1 and 2 are equally applicable to the samples that levitate in the linear range of the field (\( B_z \), Figure 2C, which is slightly weaker than the gradient we exploited in the “standard” configuration, \( B_z \)).

**Design of the Magnet Array.** Inserting magnets into the space between rows of tubes along the shorter dimension of the 96-well plate established the magnetic field for the tubes (Figure 3A). The profile of the magnetic field between every pair of like-poles facing magnets (i.e., N/N or S/S) is similar to that in the “standard” single-sample, MagLev device. We took advantage of the vertical field gradient (i.e., along the z-axis, Figure 2A, solid red line) in parallel to the faces of the magnets to levitate samples suspended in a 96-well plate. We also wished to develop this configuration of MagLev so that it would be compatible with biological samples. Two different but complementary approaches increased the strength and gradient of the magnetic field (Figure 3B, solid red line) and, thus, minimized the concentrations of paramagnetic salt used to levitate living cells and other samples sensitive to high concentrations of salts (eq 2). (i) Decreasing the size of the magnets relative to those we used in the “standard” configuration was a natural step in designing the magnet array to be compatible with the 96-well plate. The spatial profiles of the magnetic fields for permanent magnets (single or combinations) are uniformly scalable—that is, the shape and strength of the magnetic field are maintained as the absolute dimension of the field changes (e.g., compare the values of gradients in Figure 2C vs Figure 4B). This miniaturization, therefore, allows straightforward adjustment of the gradient of the magnetic field. (ii) Stacking an additional set of magnets at the bottom of the first set (Figure 4) enhanced the strength of the magnetic field in the gaps of the array. This approach of simple stacking of magnets increased the gradient of the field (from 137 T/m to 196 T/m, a factor of \( \sim 1.45 \), Figure 4B), and also increased the value of \( B_z (\partial B_z / \partial z) \) (e.g., from 28 T²/m to 116 T²/m at \( z = 1.5 \) mm, a factor of \( 4.14 \), Figure 4C). The strength of the gradient is critical in determining the concentration of paramagnetic species required to levitate a sample of a given density (eq 2). See Figures S1 and S2 for details on simulation of the magnetic fields (using Comsol).

**Choice of Paramagnetic Medium.** MagLev, as used here, requires a paramagnetic medium to levitate a diamagnetic sample. In addition, the paramagnetic medium should be compatible with (e.g., unreactive with, nondissolving, nontoxic toward) the sample to be levitated. Cost, commercial availability, volatility, and density are also important. For biological samples, only water is relevant as a solvent.

For nonbiological applications, simple paramagnetic salts (e.g., aqueous solutions of MnCl₂ or GdCl₃), hydrophilic Gd chelates (dissolved in hydrophobic solvents, such as aromatic hydrocarbons), and also paramagnetic ionic liquids can be used to levitate objects. 2,20,21

![Figure 4. Magnetic field strength in the gaps of the magnet array. (A) Schematic shows the key dimensions of a 96-well plate used in this study, and the spatial arrangement and dimensions of the magnets between the tubes. The red line indicates the magnetic field that levitates samples, and the pair of open circles mark the linear region. (B) The magnitude of \( B_z \) along the central z-axis in the gap increased by stacking a second set of magnets (represented by the white boxes in A) below the first set (represented by the filled boxes in A). The equations of linear fits to the curves within the highlighted region are \( z = 5.06B_z + 4.18 \) \( (R^2 > 0.99, \text{solid curve}) \) and \( z = 7.34B_z + 3.18 \) \( (R^2 > 0.99, \text{dashed line}) \). (C) The magnitude of \( B_z (\partial B_z / \partial z) \) increased by \( \sim 4x \) at \( z = 1.5 \) mm by stacking a second set of magnets. The jagged steps on the lines are due to the low spatial resolution we used in the simulation.](image-url)
from the scanner body, and thus, can be raised in height to accommodate the MagLev device (Figure S7). (iv) The scanner has a high optical resolution (6400 dpi, i.e., ∼4.0 μm per dot), a resolution useful to image small particles (e.g., suspended powders or clusters of cells). (v) The scanning process is fast (e.g., it took ∼10 min to scan an entire 96-well plate at a resolution of 6400 dpi). (vi) The scanner is compact, lightweight, and easily portable.

One major shortcoming in directly using the flatbed scanner to image levitated samples in the MagLev device is that the scanner has its focal plane at the flatbed, and has a limited depth of field to resolve clearly samples that are placed at a distance above the focal plane, or the flatbed. For the configuration of the MagLev device we describe in this work, we inserted mirrors in the gaps of the magnet array and between the tubes, the only space we can conveniently access to install mirrors for the tubes at ∼45° to project images of the tubes downward to the scanner. The images of the samples in the mirrors that formed are at least ∼13 mm (the height of the stacked magnets) above the flatbed of the scanner.

To solve this “out-of-focus” problem, we employed an array of relay lenses, a lens (e.g., a simple, inexpensive, biconvex plastic lens) that would form a focused image of an object on the other side of the lens, to project focused images of the samples within the tubes onto the flatbed of the scanner, the plane on which we usually place samples (e.g., a document) to be scanned.

In addition, the specific design of the scanner using a single focusing lens (or equivalent) has an oblique angle in viewing a 3D object placed on the flatbed, in a position that is laterally shifted from its central axis (c-axis, Figure S; see Figure S4B for an example using binder clips). Carefully adjusting the angles of the mirrors, and the lateral displacements of the lenses, D, with respect to the central axis of the scanner, generated focused, nonoblique views of the samples that levitated in the tubes (see Figures S3–S6 for detailed designs). Due to the particular shape of the magnetic fields between the magnets, the samples (e.g., small colored particles having the same density) levitate at the same z-coordinates, and form straight lines (in the y–z plane) parallel to the faces of the magnets (Figure 5B). Because the mirrors face the tubes in the gaps, the reflected images of the lines in the mirrors, and also the refocused images on the flatbed of the scanner, appear to the scanner as single dots (Figure 5A, B). Figure 5C shows an image of a set of four colored particles (used as density standards) that the scanner acquired. These particles appeared as single dots on the acquired image; the view to the particles behind the flatbed. For the configuration of the MagLev device we describe in this work, we inserted mirrors in the gaps of the magnet array and between the tubes, the only space we can conveniently access to install mirrors for the tubes at ∼45° to project images of the tubes downward to the scanner. The images of the samples in the mirrors that formed are at least ∼13 mm (the height of the stacked magnets) above the flatbed of the scanner.

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## RESULTS AND DISCUSSION

Selecting the Number and Dimensions of Magnets.

We chose a typical 96-well plate used for applications with polymerase chain reactions for this study because of the optical transparency of its thin-walled tubes, and also because it was easy to insert magnets between the rows of the tubes. We used the dimensions of the tubes that fitted in the wells (conically shaped tubes with a cone angle of ∼17°, a height of 13 mm, and an intertube spacing of 9 mm, Figure 4A) to select the number and size of the magnets.

We used COMSOL to simulate and evaluate the profile of the magnetic field in the gaps of the magnet array (Figures 3C, S1, and S2), and chose—based on the commercial availability of the magnets and the spatial constraints of the well plate—the following dimensions to construct the magnet array: 15 magnets of 101.6 mm × 4.8 mm × 6.4 mm (L × W × H). We stacked a second set of magnets of the same type at the bottom to further increase the strength of the magnetic field while maintaining an approximately linear magnetic field over ∼3 mm in the gaps (Figure 4B). The enhanced strength and gradient of the magnetic field are critical in decreasing the concentrations of the paramagnetic salts required to levitate living cells.

Preparing Density Standards. We usually use commercially available and highly precise (∓0.0002 g/cm³) density standards (glass beads, American Density Materials, Inc.) to calibrate the “standard” MagLev device. These beads are ∼4–5 mm in diameter (Figure 6A), too large to be directly useful to calibrate the MagLev device we describe in this study. There are two simple methods useful to calibrate the device: (i) use small particles having known densities and (ii) use hydrophobic liquids having known densities (in the form of small droplets suspended in an aqueous solution, or emulsions). We preferred method (i) to method (ii) because multiple density standards can be easily combined in a single solution, which allows convenient preparation, use, and storage of these density standards for calibration purposes.

Density standards of small (∼200 μm in diameter), colored particles are commercially available (Cospheric, LLC); these beads, however, may have a large distribution in density (e.g., ∆ρ ~ 0.1 g/cm³ for the blue particles in Figure 6B), and thus, are suboptimal to calibrate the device we describe in this study.
We used the “standard” MagLev device to fractionate the beads and improve the quality of the density standards. We improved the precision in density (that is, we narrowed the distribution in density) of these beads by up to ~8x. We could, if needed, further improve the precision of the density standards using AMPS that we have described in a separate study. Each population of the prepared particles has a spread in density ~ ± 0.01 g/cm³ around its average density (Figure 6C).

Calibrations and Reproducibility across the Array. Calibration of the MagLev device used the particles prepared in 0.100 M MnCl₂ (Figure 7) at room temperature (23 ± 1 °C). We measured the levitation heights, defined as the apparent distance D between the centroid of the standard particle(s) and the horizontal line running through the center of the view area. (This distance D measures the separation between the centroid of the particles and the center of the viewing area on the image that formed at the flatbed, and thus, does not represent the physical distance in the tube. Figure 5.) Figure 7B shows a representative calibration curve for a tube on the plate. The linear fits of D vs density for particles that levitate in individual tubes across the plate give an excellent average R² of 0.97 (N = 91 wells, Figure 7C). We excluded five wells because they either missed one or more colored beads or produced low-quality images. In another set of experiments, we performed an additional calibration using small drops of anisole (ρ = 0.993 g/cm³, stabilized by 1% Tween-20 in the suspending medium), and the combined results yielded an average R² of 0.98 across the plate. Together, these results validated the assumption of approximately linear magnetic fields in the magnet array.

Density Measurements of Simple Liquids and Irregularly Shaped Solids. Cholesterol and 3-chlorotoluene served as examples to illustrate the use of the MagLev device to measure the densities of simple liquids and irregularly shaped solids (Figure 8). Including 1% surfactant Tween-20 in the suspending medium (0.100 M MnCl₂) facilitated the dispersion of the hydrophobic compounds (particles or liquid drops) in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium. At equilibrium, the small drops of 3-chlorotoluene appeared as white clusters in the aqueous suspending medium.

Separation and Density Measurements of Mixtures of Particles and Powders. A mixture of spherical glass particles
concentrations), (iv) has a low toxicity,\(^1\) and (v) is highly transparent (it has a faint yellow color even at high \(\text{GdCl}_3\),\(^1\)) (ii) has a high solubility in water (\(1.6927 \text{ g/cm}^3\) using a densitometer) of the suspending medium (e.g., using the calibration curve in Figure 7 for that tube), and then experimentally measured the density of cholesterol, \(1.030 \pm 0.005 \text{ g/cm}^3\) (\(N = 95\) wells). The estimated density of cholesterol is \(1.067 \pm 0.008 \text{ g/cm}^3\) (\(N = 95\) wells). The literature value (\(\rho_{\text{reported}} = 1.075 \text{ g/cm}^3\)) was included for comparison.

**Figure 8.** Density measurements of a liquid and a solid. (A) We levitated small, surfactant-stabilized droplets of 3-chlorotoluene in an aqueous solution of 0.100 M \(\text{MnCl}_2\), and then experimentally measured the density using MagLev. (B) Small crystals of cholesterol, in an aqueous solution of 0.100 M \(\text{MnCl}_2\). The sample was prepared by vigorously shaking 0.5 mL of 3-chlorotoluene in 20 mL of \(\text{MnCl}_2\) solution containing 1 wt % Tween-20, and adding the sample as an emulsion to the wells using a 12-channel pipettor. The estimated density of the droplets is \(1.069 \pm 0.008 \text{ g/cm}^3\) (\(N = 95\) wells). The estimated density of 3-chlorotoluene is \(1.069 \pm 0.008 \text{ g/cm}^3\) (\(N = 95\) wells). The literature value (\(\rho_{\text{reported}} = 1.075 \text{ g/cm}^3\)) was included for comparison.

and irregularly shaped copper powder was prepared as an example to demonstrate the use of the MagLev device to perform separation and then measure the densities of its constituents of the mixture (Figure 9). Because glass and copper are more dense than typical organic materials, an aqueous solution of 3 M \(\text{DyCl}_3\) was used to levitate these samples.

\(\text{DyCl}_3\) is a suitable paramagnetic salt for this application because it (i) has a higher magnetic susceptibility than those of more commonly used paramagnetic species (e.g., \(\text{MnCl}_2\) and \(\text{GdCl}_3\)),\(^1\) (ii) has a high solubility in water (\(\sim 3.5 \text{ M}\)),\(^1\) (iii) is highly transparent (it has a faint yellow color even at high concentrations), (iv) has a low toxicity,\(^2\) and (v) is commercially available at an affordable price. (We purchased 100 g for \(\sim 46\)$. The mixture was suspended in a \(\text{DyCl}_3\) solution with 1 wt % Tween-20, and added to the MagLev device, two clearly separated clusters of particles with easily distinguishing colors (Figure 9B). We calculated the densities of the two clusters using eq \(2\), instead of establishing and using a calibration curve (because there were no easily accessible density standards for this range of density). We calculated, using the eq \(2\), the profile of \(B_z(dB_z/dz)\) along the \(z\)-axis over the linear range (e.g., using the calibration curve in Figure 7 for that tube), and then experimentally measured the density (1.6927 \(\text{ g/cm}^3\) using a densitometer) of the suspending medium (3 M \(\text{DyCl}_3\)), and also its magnetic susceptibility (1.56 \(\times 10^{-3}\), see the Supporting Information for details). We estimated the densities of the copper clusters in individual tubes (see the Supporting Information for details), and then obtained an average density of 7.7 \(\pm 0.6 \text{ g/cm}^3\) (\(N = 95\) wells). The estimated average agrees qualitatively (\(\Delta \rho = 1.3 \text{ g/cm}^3\) or \(\sim 14\%\)) with the reported value, 8.96 g/cm\(^3\).\(^2\) This discrepancy may arise from sample preparations (e.g., incomplete removal of trapped air bubbles), and we did not improve the protocol further in this study. We performed similar calculations for the clusters of the glass beads, and obtained an average density of 2.5 \(\pm 0.5 \text{ g/cm}^3\) (\(N = 95\) wells), which agrees with the value, 2.40 \(\pm 0.04 \text{ g/cm}^3\), for the same type of glass beads we measured in a separate study using tilted MagLev.\(^1\)

**Density Measurements of Erythrocytes.** Erythrocytes served as an example to demonstrate the use of the device to levitate and measure densities of biological particles (Figure 10). In this demonstration, the biocompatible paramagnetic chelate, gadobutrol, was used to levitate erythrocytes. Density...
standards (the same particles as we described in Figure 6) were included in the same suspending medium to calibrate the system, and thus to calculate the density of the erythrocyte cluster that located between the two standard particles. No surfactant Tween-20 was used in this experiment (we washed the beads with PBS); enough beads were used so that the majority of the wells had both types of particles that levitated in the medium. (These hydrophobic beads tend to trap at the liquid−air interface in the absence of a surfactant, and we did not optimize the experimental protocol further in this study.) We estimated the density of the erythrocytes to be $1.10 \pm 0.03$ g/cm$^3$ ($N = 93$ wells), which agrees well with the values ($\sim 1.11$ g/cm$^3$) reported in the literature.$^{2,3}$

**Determination of the Arrhenius Activation Energy of a Reaction on a Solid Support.** As the final demonstration, we used the MagLev device to monitor the progress of a coupling reaction of 2,5-diiodobenzoic acid with leucine-functionalized Wang resin (porous polymer beads, 74−149 μm in diameter). MagLev, as we have demonstrated previously using the “standard”, single-sample configuration,$^{27}$ is a particularly suitable tool with which to monitor conveniently certain types of chemical reactions on solid supports. The coupling reactions, as described in this study, were carried out in a small volume (5 mL) under controlled temperatures (23.6, 7.6, −0.4, and −13.0 °C, see the Supporting Information about the specific cooling baths). Small aliquots (0.5 mL) were sampled periodically during the reaction.

We determined empirically the composition of the suspension medium (11 mM GdCl$_3$ and 0.7 M ZnBr$_2$ in dimethylformamide) to levitate the polymer beads in the device at room temperature (23 ± 1 °C), such that the densities of the unreacted (1.04 g/cm$^3$), experimentally determined using the “standard” MagLev and fully converted (1.12 g/cm$^3$) beads spanned approximately the full range of density (Figure 11B). The levitation height of the beads enabled the calculation of the fraction of conversion of the amine present on the beads, and also of the rate constants at different temperatures (Figure 11C, see Supporting Information for details on calculation). We determined the Arrhenius activation of this reaction (Figure 11D) to be 55 kJ/mol, which agrees with a reported value (64 kJ/mol, a relative difference of 14%).$^{28}$

**CONCLUSION**

MagLev, as we and others have developed it, is an easily accessible technique with which to separate and measure densities of diamagnetic materials using a paramagnetic suspending medium and inexpensive permanent magnets. The existing methods of MagLev, however, lack a significant analytical capability—high-throughput separation, analysis, and/or density measurements of materials—and this limitation has slowed the development of this technique.

This paper describes the design of a re-engineered configuration that combines the simplicity of MagLev with a broadly available imaging system (a flatbed scanner and simple optics) to levitate and image samples in a paramagnetic medium using 96-well plates. This integrated analytical system delivers the capability of high-throughput analysis in a format that is simple, inexpensive, and broadly applicable to a variety of types of samples—including biological cells—having a size in the range of μm to sub-mm (e.g., particles, powders, emulsions, and living cells). The limitations of the technique are (i) its incompatibility with samples having a size of mm or above and (ii) the use of plastic 96-well plates, which excludes the uses of suspending media that may dissolve the materials of the plates (e.g., polypropylene or polystyrene).

This integrated analytical system is broadly applicable to areas in which density could be exploited as a useful physical parameter, and in which there are needs for simplicity, affordability, and, particularly, the capability to monitor densities of samples in a high-throughput format. This system will broaden density-based applications available to MagLev; it may be particularly useful to (i) materials chemistry to separate, analyze, and/or identify materials, and to monitor physical and/or chemical changes of materials over time,$^4$ (ii) forensic science and other areas that deal extensively with various sorts of materials (e.g., analysis of trace evidence, and separation/identification of small minerals for geological applications), (iii)
analytical science to develop broadly useful and easily accessible density-based assays,3,29 and (iv) biological and medical fields to measure and monitor changes in density associated with cellular activities and/or physiological conditions.6,8 The optical system and design used may also be valuable for other parallel measurements using similar formats.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b01283.

Selection of the number and dimensions of magnets; design of an apparatus to image samples that levitate in a 96-well plate using a scanner; assembly of the device; analysis of images; estimation of experimental uncertainty; determination of the magnetic susceptibility of a paramagnetic medium; experimental procedures to perform the coupling reaction of 2,5-diiodobenzoic acid with leucine-functionalized Wang resin; and determination of the Arrhenius activation energy of a reaction on a solid support (PDF).

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**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

This work was funded by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Materials Sciences and Engineering under Award Number ER45852. Specifically, the DOE grant supported the design of the MagLev device and its use to perform density measurements. S.G. and D.J.P. acknowledge salary support from DOE (Award Number ER45852). Y. Wang and N. Deshler thank the NSF-supported REU program (Grant number: DMR-1420570) for support.

**REFERENCES**

High-throughput Density Measurement Using Magnetic Levitation

Supplemental Information

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Materials

Blood samples were purchased from Blood Research Components, LLC.

Selecting the number and dimensions of magnets

Simulation in COMSOL® shows that a minimum number of 15 magnets and a minimum length of ~101.6 mm (four inches) of the magnets are required to create a nearly uniform magnetic field in all 12 gaps to accommodate all 12 columns of tubes on a 96-well plate. We used an array of 15 magnets of 101.6 mm × 4.8 mm × 6.4 mm (L×W×H)—half of the final magnet array we used in this study—as an example to illustrate the design.

First, we found that the profile of the magnetic field in the outermost gaps deviates slightly from those in the middle, which are virtually indistinguishable. For example, the strength of the field along y-axis (along the longest dimension of the magnets) at both z=H_1/2 and z=H_1 (Figure S1A, plane b, green lines. H_1 is the height of the magnet, and z=0 is defined at its bottom face) in the middle of the outermost gaps 7 and 7’ deviates from those in other gaps (Figures S1C and S1E). Similarly, the z-component of the magnetic field along the vertical centerlines of the gaps (Figure S1A, white lines on plane a) in the outermost gaps deviates from those in other gaps (Figures S1B and S1D). While the outermost gaps are similarly functional to levitate objects, we simply excluded them in this study for simplicity (e.g., to avoid having to carry out separate calibrations for density measurements).

Second, we found that a minimum length of 101.6 mm (four inches) of a magnet is required to create a uniform profile of the magnetic field in the gaps along the length of the magnet (represented by the flat profiles of the field along the y-axis in the middle
A. An array of single magnets

B. Magnetic field on plane a

C. Magnetic field on plane b in the gap

D. Graph showing the magnetic field B with distance in millimeters:
   - Gap 1
   - Gap 2
   - Gap 3
   - Gap 4
   - Gap 5
   - Gap 6
   - Gap 7
   Middle line in the gap

E. Graph showing B profiles along the green lines at z = H_z in A.
   - Gap 1'
   - Gap 2'
   - Gap 3'
   - Gap 4'
   - Gap 5'
   - Gap 6'
   - Gap 7'
   B profiles along the same green lines but at z = H_z/2.
Figure S1 Selection of the number and dimensions of the magnets to create an identical profile of magnetic field for all the tubes on a 96-well plate. (A) A schematic of the spatial arrangement of the magnets. In this schematic, we showed only half of the array (only the top magnets) that we constructed experimentally. Plane a on the x-z plane cuts through the magnet array at half its length (L/2 along y-axis). Plane b on the y-z plane sits in the middle of the 1’ gap. The red lines represent the vertical symmetric axes of the gaps. The green lines run along the y-axis in the middle of the gaps, and (as shown) are on the same plane as the top faces of the magnets. (B) The profile of magnetic field strength (represented by the absolute magnitude of the field, ||B||) on plane a. (C) The profile of magnetic field strength (||B||) on plane b. The dashed box indicates the magnets in the y-z plane. (D) Bz along the white lines in (A). (E) ||B|| along the green lines in (A) at two heights of z=H1 (as shown in (A)) and z=H1/2. The grayed region represents the length (L=4” or ~101.6 mm) of the magnets.
segments, Figure S1E). The magnetic field is not uniform—as expected—towards the edge of the magnets. The uniform profile of the field is important to ensure that all eight tubes in a single column on the plate (which spans ~63 mm) experience indistinguishable magnetic fields.

Third, we optimized the specific dimensions of the magnets—the width (W) and height (H). Eq 2 in the main text suggests that for a given concentration of paramagnetic medium (and thus a fixed value of the magnetic susceptibility of the suspending medium), the measurable range of density is determined by the characteristics of the magnetic field as indicated by the $B_z (dB_z/dz)$ term; we, therefore, evaluated its dependence on the dimensions of the magnets.

In the simulation, we placed the origin of the z-axis at the bottom of the tube to maximize the use of the space between the magnets for density measurements. We then set the bottom faces of the magnets at $z=0$ and swept the height of the magnets $H_1$—and the width of the magnets changes accordingly as the magnets adapt to the tubes on the plate. We quantitatively evaluated the influence of height of the magnets on the $z$-component of the field $B_z$ and the term $B_z (dB_z/dz)$.

Figure S2B and S2C show that as the height of the magnets increases from 2 mm to 12 mm, the $z$-component of the magnetic field $B_z$ increases to reach a maximum at $H_1$~6 mm while $B_z (dB_z/dz)$ reaches its maximum earlier at $H_1$~4 mm. As $H_1$ increases beyond ~8 mm, neither $B_z$ nor $B_z (dB_z/dz)$ is linear. Given the commercial availability of magnets (Long and thin NdFeB magnets are brittle and susceptible to mechanical breakage, and are only available in limited selection of sizes and shapes even for customized magnets), and the physical dimensions of the 96-well plate, we used 15
Figure S2 Simulation-guided selection of the dimensions of the magnets (A) A schematic of the spatial arrangement of the magnets and the tubes on a 96-well plate that we used for the simulation. In the simulation, we set the origin of the z-axis at the bottom of the tube, and then fix the bottom face of the (top) magnet at z=0, while allowing the height and width of the magnet to change according to the contour of the external surface of the tube. (B) and (C). B<sub>z</sub> and the derived term B<sub>z</sub> × (dB<sub>z</sub>/dz) along the central line (the red line on the left in A) as the height of the magnets (H<sub>1</sub>) varies. (D) and (E) B<sub>z</sub> and the derived term B<sub>z</sub> × (dB<sub>z</sub>/dz) along the central line (the red line on the right in A) as the height of the bottom magnets (H<sub>2</sub>) varies while maintaining H<sub>1</sub>=6.4 mm.
magnets of $H_1 = 6.4$ mm to generate uniform magnetic fields for all of the tubes on a plate, while maximizing both the working distance and the gradient of the magnetic fields.

Lastly, we stacked a second set of magnets at the bottom of the first set to increase the strength of the magnetic field further, and thus, to reduce the concentration of the paramagnetic species in the medium required to levitate samples. We exploited the strong magnetic field around the boundary where two opposite poles of the magnets meet as shown in Figure 2C in the main text. In the simulation, we used the same width and length for the second set of magnets, and swept its height $H_2$. The $B_z$ at $z=0$ in the gap (Figure S2D) clearly increases when the height of the second set of magnets $H_2$ increases from zero to $H_2 = H_1$, and then quickly plateaus beyond $H_2 > H_1$. We, therefore, simply selected $H_2 = H_1$ for the final configuration we describe in this study. We estimated that this simple approach of stacking magnets increased $B_z (dB_z/dz)$ by $\sim 4\times$ (at $z=1.5$ mm, Figure S2E)—that is equivalently we may use $\sim 4\times$ diluted paramagnetic medium to levitate samples of the same density (eq 2 in the main text).

**Design an apparatus to image samples that levitate in a 96-well plate using a scanner**

We used a flatbed scanner to acquire images of the levitated samples in a 96-well plate, and designed an apparatus (including a flatbed scanner and a simple interface) using mirrors and relay lenses to project focused images of the levitated samples to the scanner bed. Figure S3 shows the design of the apparatus we implemented in this study, and Table S1 lists the dimensions for the spatial arrangement of the key components. Logistically, we first used simple models (based on ray diagrams) to guide the selection of specific sets of parameters for the key components (e.g. angles of mirrors,
Figure S3. Spatial arrangement of magnets, mirrors and lenses used to levitate and image samples (three colored beads, as an example) in paramagnetic media in a 96-well plate.
Table S1 Spatial arrangement of mirrors and lenses

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral shift of the mirror ($D$, mm)</td>
<td>-24.0</td>
<td>-15.0</td>
<td>-6.0</td>
<td>3.0</td>
<td>12.0</td>
<td>21.0</td>
<td>30.0</td>
<td>39.0</td>
</tr>
<tr>
<td>Angle of the mirror</td>
<td>40.2°</td>
<td>42.0°</td>
<td>43.8°</td>
<td>45.6°</td>
<td>47.4°</td>
<td>49.2°</td>
<td>51.0°</td>
<td>52.8°</td>
</tr>
<tr>
<td>Lateral shift of the lens ($x_p$, mm)</td>
<td>-23.4</td>
<td>-15.0</td>
<td>-6.5</td>
<td>1.8</td>
<td>10.2</td>
<td>18.5</td>
<td>27.2</td>
<td>35.6</td>
</tr>
</tbody>
</table>

*note: See Figure S3 for assignment of Tube No. All values are reported with respect to the central axis of the scanner, and the direction of the lateral shift of the illumination is designated as “positive”.*
and lateral shifts of relay lens with respect to the tubes), and then optimized these
dimensions empirically to arrive at the final values. We describe the specific
configurations for the three key parts (the scanner, the mirrors, and the lens) of the
apparatus in detail.

(1) The scanner

We used the scanner (Epson®, Perfection, V600), and its associated software
(specifically, the “film” mode) to control and acquire the images. The scanner has a
flatbed with a width 223 mm, and, in its closable lid, a LED that provides a uniform, line
illumination (83 mm). The lid is physically attached to the body of the scanner (through a
cable), but can be raised from the body of the scanner for a maximum distance of ~65
mm—a distance large enough to accommodate the MagLev device. The LED traverses,
during scanning, along the central axis of the scanner, and illuminates a strip that
overlaps the central axis of the scanner with a lateral shift of ~7.5 mm in the orthogonal
direction to the central axis. The scanner and the software records the central region of
the strip with a width of ~60 mm.

When the scanner is used to image 3D-objects (e.g. the MagLev device), it
provides an oblique view, that is objects placed further away from the central axis of the
scanner appear to be tilted toward the axis (Figure S4B). This observation probably
originates from the optics of the scanner, and is not a surprise given the fact that the
scanner is usually optimized to image thin, 2D sheets. We used a simple convex lens to
model the optics internal to the scanner (which we presume is based on a similar design),
and estimated the critical parameter (~240 mm, based on the simple model as we
described in Figure S4), the distance of physical separation between the flatbed and the
Figure S4. Estimation of the physical separation of the lens and flatbed of the scanner (A) a schematic of a simple optical system to model the optics internal to the scanner. An object with a height of \( h \) is placed on the flatbed at a distance of \( D \) to the central axis of the scanner, and has a perceived length of \( x \) in the image acquired by the scanner. We used similar triangle to calculate the physical separation, \( h \), of the flatbed and the lens. (B) Four binder clips (\( h=32 \text{ mm} \)), along with a ruler, were placed on the scanner at various distances (marks on the ruler: 2.0 cm, 5.0 cm, 8.0 cm and 11.2 cm) to the central axis (dashed white line).
lens of the scanner. We used this parameter to design and guide the spatial placement of mirrors and lenses.

(2) The mirrors

All mirrors were inserted in the gaps of the magnet array of at ~45° facing downward to project images of the individual tubes. In each of the 12 gaps, we inserted a total of eight mirrors at an inter-mirror separation of 9 mm, positioned the centers of the mirrors at the half height of the top magnets, and finally aligned the row of mirrors symmetrically about the axis of illumination. Because of the axis of illumination is laterally shifted by +7.5 mm (we defined the shifts in the direction of the illumination axis as positive values), the coordinates for the centers of the mirrors are thus the following: 39, 30, 21, 12, 3, -6, -15, -24 (mm).

We finely tuned the angles of the mirrors for tubes situated at different distances to the central axis of the scanner to provide a non-oblique view of the sample that levitate in these tubes. Because of the shape of the magnetic field, the samples, e.g., small particles, form horizontal lines within the tubes. We adjusted the spatial arrangements of the mirrors and lenses such that the lines appeared as single dots on the acquired images.

We developed a simple model (Figure S5) to estimate the angle at which the axis of the tube in the reflected image is perpendicular to the line defined by the centers of the mirror and the lens of the scanner, and used this angle to provide an non-oblique view of the samples in the tube.

(3) The lens
Figure S5. Determination of the optimal angles of the mirrors to provide a “head-on” view of levitating objects. The center of the mirror is positioned at $4f$ above the flatbed of the scanner. $f$ is the focal length of the relay lens, and $H$ is the distance between the flatbed and the lens of the scanner. A relay lens (not shown), when placed at a distance of $2f$ below the tube, will project an image of the tube to the flatbed of the scanner at a distance of $2f$ with a 1:1 magnification. $D$ is the distance of the mirror to the central axis.

\[ \varphi = 45^\circ - \frac{1}{2} \arctan \left( \frac{D}{H+4f} \right) \]
We placed relay lenses below the mirrors to project images of the tubes to the flatbed of the scanner. We shifted the lenses toward the central axis of the scanner such that the central portion of the tubes became visible to the scanner. We used the following model (Figure S6) to estimate the lateral shifts of the lenses with respect to the central axis, and used these estimates (for a total of eight lenses for a single column of tubes on the plate) as the initial set of values to optimize the lateral shifts of the lenses.

**Assembly of the device**

We used 3D-printed plastic parts to house the magnets and assemble the supporting components (e.g. mirrors and lenses). Figure S7 depicts the major components in the fully assembled device.

**Analysis of Images**

We processed images of each well with custom software to determine the positions of the levitated samples relative to the center of the viewing circle. We calibrated each well based on the measured locations of density standards (~200 μm colored particles). We then measured the densities of samples not used during calibration and compared our experimentally-measured densities with literature values. The software identified and segmented samples based on either a distinguishing color or a change in contrast at the boundary of the samples.

We identified the center of each viewing circle by converting an image of the well to binary with a threshold value of 0.047 using the im2bw() function in Matlab and computing the centroid of the largest connected region.
Figure S6. Estimation of the lateral shifts of the lenses, $x_p$, with respect to the central axis of the scanner. $f$ is the focal length of the relay lens.

$x_p = \frac{D(H + 2f)}{H + 4f}$
Figure S7. Exploded and collapsed views of the final assembled device.
We identified samples with colors clearly distinguishable from the background primarily based on their hue value. This was the case for all four of the density standards that we used for calibration (Figure S8). Occasionally, the background may also show a faint color similar to the particles (it was caused by both the fluorescence that these dyed particles emitted under the conditions we carried out the experiment, and the natural color of the plastic housing we used.); the color, however, did not interfere with the determination of the positions of these particles. Cut-off hue values and other specifications are detailed in Table S2.

Several of the samples that we measured experimentally lacked a distinguishing color; we were not able to identify the locations of these samples using the procedures as described above. Instead, we used edge detection to determine the location of these samples (Figure S9).

We analyzed the spread in density of samples of red blood cells by fitting a normal distribution to the density distribution of the cells (Figure S10). The density distribution was measured experimentally by comparing with an in-situ calibration performed by fitting the measured locations of density standards (the green and blue particles).

We described the preceding image processing and analysis operations on a per-well basis. To evaluate the entire 96-well plate, we ran these operations in a loop over an image of the entire plate, where the image of the entire plate was spliced into 96 sections (8×12), each of which was analyzed serially.
Figure S8. Image processing of colored particles in a well to determine their locations relative to the well centroid.
Table S2. Identifiers and constraints used to segment colored particles from images.

<table>
<thead>
<tr>
<th>Color</th>
<th>Identifier 1</th>
<th>Constraint on 1</th>
<th>Identifier 2</th>
<th>Constraint on 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>Hue</td>
<td>0.17 &lt; H &lt; 0.45</td>
<td>Value</td>
<td>H &gt; 0.97</td>
</tr>
<tr>
<td>Purple</td>
<td>Hue</td>
<td>0.80 &lt; H &lt; 0.98</td>
<td>Value</td>
<td>H &gt; 0.60</td>
</tr>
<tr>
<td>Red</td>
<td>Hue</td>
<td>0.01 &lt; H &lt; 0.07</td>
<td>Value</td>
<td>H &gt; 0.75</td>
</tr>
<tr>
<td>Blue</td>
<td>Hue</td>
<td>0.45 &lt; H &lt; 0.65</td>
<td>Red Channel</td>
<td>R &lt; 0.35</td>
</tr>
</tbody>
</table>
Figure S9. Processing of an image of cholesterol suspended in a well using an edge detection algorithm. Starting from the original image (a), the value channel of the hue-saturation-value representation is extracted (b) and blurred with a Gaussian filter (c). Edges are detected with the Canny edge detection algorithm (d), and regions outside of the well are cropped (e). Finally, artefactual edges under a threshold length are removed (f), the remaining edges are dilated (g), and the region of interest within the edges is filled as a convex hull of the edge pixels.
Figure S10. The spread in density for a sample of red blood cells was determined by first calibrating the density in-situ using the density standards (a, the green and blue particles), and then fitting a normal distribution to the density distribution of the cells (b). For this sample, the estimated mean density of the red blood cells was 1.11 g/cm$^3$, and the spread, represented by the standard deviation, was $\sim 0.01$ g/cm$^3$. 
Estimation of the uncertainty of measurements for samples in single tubes using calibration curves

We used a set of four colored particles to calibrate the density measurements in individual wells, and here, estimated the uncertainty in the estimated values of densities of samples using the calibration curves if we treat single tubes as independent measurements.

Eq S1 gives the equation for the best-fit for the plot of density vs. the distance D of the colored particles to the center of the viewing area. We used four pairs of data \((D_i, \rho_i)\) (i=1-4) to establish the calibration curve for each tube. For this discussion, D (unitless) simply represents the number of pixels on the image, and is negative when the centroid of the particles are below the center of the viewing area on the image. (We did not convert the number of pixels to physical distances for this example.) \(\rho\) (kg/m³) is the average density of the particles.

\[
D = m\rho + b
\]  

(S1)

For a sample with unknown density (e.g., a cluster of particles), we first determine its D, and then insert it to eq S1 to calculate its density.

Eq S2 gives the uncertainty, \(\delta\rho\), in the estimate of density using the calibration curve, Eq S1.\(^1\) In eq S2, \(S_D\) is the standard deviation of the vertical distances on the calibration curve between the D-coordinates of the colored particles and the best-fit curve, \(|m|\) is the absolute value of the slope, k is the number of replicate measurements of the unknown sample, n is the number of data points to establish the calibration curve, \(\bar{D}\) is the average of the D for the colored particles, \(\bar{\rho}\) is the average of the densities for the
colored particles, and $D$ is the distance between the centroid of the sample to the center of the viewing circle.

$$\delta \rho = \frac{s \rho}{m} \sqrt{\frac{1}{k} + \frac{1}{n} + \frac{(D-B)^2}{m^2 \Sigma (\rho_i - \bar{\rho})^2}}$$

(S2)

We applied eqs S1 and S2 to estimate the density, and its associated uncertainty, of a sample of 3-chlorotoluene (a single measurement, $k = 1$) in the tube shown in Figure 6A, and obtained $1.068 \pm 0.006$ g/cm$^3$.

**Experimental determination of the magnetic susceptibility of a paramagnetic medium**

We determined experimentally the magnetic susceptibility of an aqueous solution of nominal 3 M DyCl$_3$. We first diluted the concentrated solution by 6× to yield an aqueous solution of nominal 0.5 M DyCl$_3$, and then levitated a set of glass beads (1.0200, 1.0630, 1.1100, and 1.1550 g/cm$^3$) with precisely known densities ($\pm 0.0002$ g/cm$^3$) using the standard MagLev device. We used a ruler with a minimal division of 1 mm (read to ±0.1 mm) to measure the levitation heights of the beads.

We plotted the density vs. $h$, and performed linear fit, which yielded eq S3:

$$\rho = -6663(\pm 187)h + 1267(\pm 5)$$

(S3)

In eq S3, $\rho$ ($kg/m^3$) is the density of a sample that levitated at a distance of $h$ ($m$) to the surface of the bottom magnet. The slope is presented as best-fit value ±standard deviation of the best-fit value.

Eqs S4-6 give the formulas for $\rho$ vs. $h$ using the standard MagLev device.$^2$

$$\rho = \alpha h + \beta$$

(S4)
\[ \alpha = \frac{(\chi_s - \chi_m)4B_0^2}{g\mu_0 d^2} \]  

\[ \beta = \rho_m - \frac{(\chi_s - \chi_m)2B_0^2}{gH_0 d^2} \]  

In eqs S5 and S6, \( \chi_s \) (unitless) is the magnetic susceptibility of the sample, \( \chi_m \) (unitless) is the magnetic susceptibility of the paramagnetic medium, \( B_0 \) (T) is the maximum strength of the linear magnetic field along central axis in the gap between the two facing magnets, \( g \) (9.8 m/s\(^2\)) is the constant of gravitational acceleration, \( \mu_0 \) (\(4\pi \times 10^{-7}\) N\(\cdot\)A\(^{-2}\)) is the magnetic permeability of the free space, \( d \) (m) is the distance of separation of the two magnets, \( \rho_m \) (kg/m\(^3\)) is the density of the paramagnetic medium.

We used eqs S3 and S5 to calculate the magnetic susceptibility of the solution, \( \chi_m \), and the part that contributed by the DyCl\(_3\), \( \chi_{DyCl_3} \), using a simple model in which the glass beads (we used \( \rho_{glass} = 1.1100 \text{ g/cm}^3 \) for this calculation) consist of glass (\( \rho_{glass} \approx 2.4 \text{ g/cm}^3 \), \( \chi_{glass} = -1.39 \times 10^{-5} \)), and an air pocket (we assumed zero density or magnetic susceptibility).

\[ \chi_{DyCl_3} = \chi_m - \chi_{H_2O} \]  

To calculate the \( \chi_{DyCl_3} \), we used the experimentally measured values for \( B_0 \) (0.385 T), and \( d \) (45.0 mm). We estimated the magnetic susceptibility of the glass bead, \( \chi_s \), using a simple model in which the glass beads consist of glass (\( \rho_{glass} \approx 2.4 \text{ g/cm}^3 \), \( \chi_{glass} = -1.39 \times 10^{-5} \)), and an air pocket (we assumed zero density or magnetic susceptibility).

\[ \chi_{bead} = \chi_{glass} \frac{V_{glass}}{V_{bead}} = \chi_{glass} \frac{\rho_{glass}}{\rho_{bead}} = -3.0 \times 10^{-5} \]  

In eq S8, \( \chi_{H_2O} \) is the magnetic susceptibility of water (\(-9.0 \times 10^{-6}\)).
We assumed that the major uncertainty in estimating the $\chi_{\text{DyCl}_3}$ stemmed from the uncertainty of the linear fit, and estimated the magnetic susceptibility of the diluted DyCl$_3$ solution, $\chi'_{\text{DyCl}_3}$:

$$\chi'_{\text{DyCl}_3} = (2.60 \pm 0.08) \times 10^{-4} \quad (S10)$$

We, therefore, determined the magnetic susceptibility of the original solution of nominal 3 M DyCl$_3$:

$$\chi_{\text{DyCl}_3} = (2.60 \pm 0.08) \times 10^{-4} \times 6 - 9.0 \times 10^{-6} = (1.56 \pm 0.05) \times 10^{-3} \quad (S11)$$

**Calculation of the density of a cluster of particles**

We first estimated the magnitude of the $B_x (dB_x/dz)$ at distance D (with respect to the center of the viewing circle, Figure 6A) using the calibration curves established by the small, colored particles.

Eq S12 gives the equation of the linear fit for the colored particles in a tube.

$$D = m \rho + b \quad (S12)$$

Eq S13 is the same equation, eq 2, that we described in the main text. This equation allows us to estimate the value of $B_x (dB_x/dz)$ at the position a sample levitates (e.g., density particles having a known density and a magnetic susceptibility) in a paramagnetic medium with a known density and a magnetic susceptibility.

$$\rho = \frac{(x_s - x_m)}{\mu_0 g} \left( B_x \frac{dB_x}{dz} \right) + \rho_m \quad (S13)$$

We combined eqs S12 and S13 to give eqs S14-17 that we can use to estimate the value of $B_x (dB_x/dz)$ at any distance D in the linear range we characterized using the colored particles.
\[
\frac{B_z}{\partial z} = \frac{\mu_0 g}{m(\chi_s - \chi_m)} D - \frac{\mu_0 g(b/m + \rho_m)}{(\chi_s - \chi_m)} \quad (S14)
\]

\[
K_1 = \frac{\mu_0 g}{m(\chi_s - \chi_m)} \quad (S15)
\]

\[
K_2 = \frac{\mu_0 g(b/m + \rho_m)}{(\chi_s - \chi_m)} \quad (S16)
\]

\[
B_z \frac{\partial B_z}{\partial z} = K_1 D - K_2 \quad (S17)
\]

We calculated the values of \(K_1\) and \(K_2\) for each tube using the calibration curve that we constructed using the set of four, colored particles (1.03, 1.06, 1.10, 1.13 g/cm\(^3\)). In this experiment, we used an aqueous solution of 0.100 M MnCl\(_2\) (We prepared this solution from a stock solution, 3.000±0.001 M) to levitate the particles, and calculated its density and magnetic susceptibility using the formula as we described elsewhere in detail:\(^2\)

\[
\rho_m = 1008.1 \text{ kg/m}^3
\]

\[
\chi_m = 9.58 \times 10^{-6}
\]

The small density particles we used are based on polyethylene, and they have a magnetic susceptibility \(\chi_s\) of \(-9.50 \times 10^{-6}\).\(^4\)

We estimated \(K_1\) and \(K_2\) for each individual tube. We combined individual values of \(K_1\) or \(K_2\) across the plate, and obtained the averages for \(K_1 = 0.28\pm0.03\) (mean±SD, T\(^2\)/m), and \(K_2 = 31\pm14\) (mean±SD, T\(^2\)/m).

We next determined the centroid of a cluster of the copper or glass particles that levitated in an aqueous solution of 3 M DyCl\(_3\), and used eq S17 (and its associated pair of \(K_1\) and \(K_2\)) to estimate the value of \(B_z(dB_z/dz)\) at that distance \(D\).
We finally inserted the estimated value of $B_d(dB_d/dz)$, the experimentally measured density of the DyCl$_3$ solution (1.6927 g/cm$^3$), and the estimated magnetic susceptibility of the DyCl$_3$ solution ($1.56 \times 10^{-3}$, eq S11), to calculate the density of the cluster of the copper or glass particles. In this calculation, we neglected the magnetic susceptibility of the sample (i.e., the copper and glass particles) because they are negligible compared to the magnetic susceptibility ($1.56 \times 10^{-3}$) of the DyCl$_3$ solution that we used to levitate the samples.

We obtained the averages of the estimated densities across the plate for the sample of copper powder (7.7±0.6 g/cm$^3$, N=95 wells) and for the sample of glass particles (2.4±0.4 g/cm$^3$, N=95 wells).

Experimental procedures to perform the coupling reaction of 2,5-diiodobenzoic acid with leucine-functionalized Wang resin

We adapted experimental protocols from previous studies,$^5,6$ and made minor modifications. Fmoc-Leu-Wang resin (0.030 g, 100-200 mesh, 0.68 mmol –NH$_2$/g resin) was placed a glass vial, and dimethylformamide (1.5 mL) and piperidine (0.5 mL) were added. The mixture was allowed to react under continuous stirring (400 rpm) for 30 min at room temperature (23±1 ºC). The resin was filtered using a disposable chromatography column (Biorad #1731-1550), rinsed three times with dimethylformamide (2 mL), and transferred to a test tube containing dimethylformamide (5 mL) and a stir bar. The temperature of the mixture was controlled using the following cooling baths, and monitored during the reaction using a digital thermocouple: ethylene glycol/dry ice for −13.0 ºC (experimentally measured), water/ice for −0.4 ºC, cyclohexane/dry ice for 7.6
°C, and air for 23.6 °C. 2,5-diiodobenzoic acid (0.065 g, 0.18 mmol), O-(Benzotriazol-1-
yl)-N,N,N’,N’-tetramethyluronium hexafluorophosphate (0.065 g, 0.17 mmol), and N,N-
diethylisopropylamine (0.030 mL, 0.24 mmol) were added sequentially to the mixture to
initiate the reaction. At specific time points (time interval ranged from 0.5 min at 23.6 °C
to 5 min at −13.0 °C), an aliquot (0.25 mL) was sampled, and the resin was rinsed
immediately with dimethylformamide (2 mL × 3) on a chromatography columns to
remove the reactant. The resin was finally suspended in dimethylformamide (0.5 mL)
containing GdCl₃ (11 mM) and ZnBr₂ (0.7 M) ready for density measurements in the
MagLev device.

**Determination of the Arrhenius activation energy of a reaction on a solid support**

The initial concentration of free amine presented on the resin was calculated using
eq S18.

\[
[-\text{NH}_2]_{0\%\text{conversion}} = \left(\frac{0.68 \text{ mmol}}{g}\right) \left(\frac{1.04 \text{ g}}{\text{cm}^3}\right) = 0.71 \text{ M} \quad (S18)
\]

In eq S18, 0.68 mmol/g resin is the loading of amine on the resin, 1.04 g/cm³ is the
density of the resin (porous polystyrene beads, determined independently using MagLev
and a suspension medium of GdCl₃ and dimethylformamide).

The concentration of the unreacted amine during an experiment was estimated
using eq S19

\[
[-\text{NH}_2]_{\text{experiment}} = [-\text{NH}_2]_{0\%\text{conversion}} \left(\frac{z_{\text{experiment}} - z_{100\%\text{conversion}}}{z_{0\%\text{conversion}} - z_{100\%\text{conversion}}}\right) \quad (S19)
\]
In eq S19, $z_{\text{experiment}}$ is the z-coordinate of the beads at a given time during a reaction, $z_{0\%\text{conversion}}$ is the z-coordinate of the unreacted beads, and $z_{100\%\text{conversion}}$ is the z-coordinate of the fully converted beads.

This reaction is a first-order reaction for the amine present on the beads,\textsuperscript{5,6} all other participating reactants (i.e. O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate and N,N-diethylisopropylamine) were in excess (~10 fold), and we assumed their concentrations remained unchanged throughout the reaction. The rate of reaction, therefore, can be expressed as eq S20.

$$- \frac{d[-\text{NH}_2]}{dt} = k[-\text{NH}_2] \quad (S20)$$

$$\log([-\text{NH}_2]) = -0.434kt + \log([-\text{NH}_2]_{0\%\text{conversion}})$$

In eq S20, $k$ is the rate constant observed experimentally. We plotted $\log([-\text{NH}_2])$ vs. time to determine the rate constants under different temperatures.

To determine the Arrhenius activation energy for this reaction, we plotted $\log(k)$ vs. $1/T$ according to eqs S21 and S22.

$$k = Ae^{-\frac{E_a}{RT}} \quad (S21)$$

$$\log(k) = -\frac{0.434E_a}{R}\left(\frac{1}{T}\right) + \log(A) \quad (S22)$$

In eqs S21 and S22, $E_a$ is the Arrhenius activation energy, $A$ is the pre-exponential factor, and $R$ is the gas constant (8.31 J mol$^{-1}$ K$^{-1}$).
References


