# Efficient Sample Preparation from Complex Biological Samples Using a Sliding Lid for Immobilized Droplet Extractions

Benjamin P. Casavant,[‡] David J. Guckenberger,[‡] David J. Beebe, and Scott M. Berry*

Department of Biomedical Engineering, Wisconsin Institutes for Medical Research, University of Wisconsin, Madison, Wisconsin 53705, United States

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ABSTRACT: Sample preparation is a major bottleneck in many biological processes. Paramagnetic particles (PMPs) are a ubiquitous method for isolating analytes of interest from biological samples and are used for their ability to thoroughly sample a solution and be easily collected with a magnet. There are three main methods by which PMPs are used for sample preparation: (1) removal of fluid from the analyte-bound PMPs, (2) removal of analyte-bound PMPs from the solution, and (3) removal of the substrate (with immobilized analyte-bound PMPs). In this paper, we explore the third and least studied method for PMP-based sample preparation using a platform termed Sliding Lid for Immobilized Droplet Extractions (SLIDE). SLIDE leverages principles of surface tension and patterned hydrophobicity to create a simple-to-operate platform for sample isolation (cells, DNA, RNA, protein) and preparation (cell staining) without the need for time-intensive wash steps, use of immiscible fluids, or precise pinning geometries. Compared to other standard isolation protocols using PMPs, SLIDE is able to perform rapid sample preparation with low (0.6%) carryover of contaminants from the original sample. The natural recirculation occurring within the pinned droplets of SLIDE make possible the performance of multistep cell staining protocols within the SLIDE by simply resting the lid over the various sample droplets. SLIDE demonstrates a simple easy to use platform for sample preparation on a range of complex biological samples.
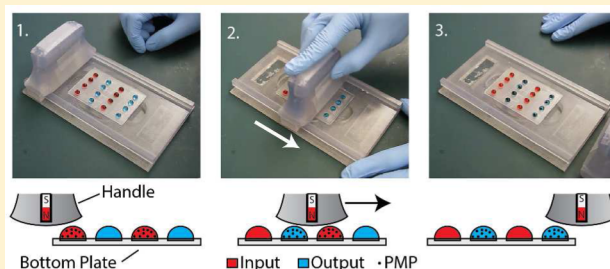
M ethods for isolating DNA, RNA, and protein from biological samples are central to molecular biology. However, these methods are often overlooked as new assays are developed for the biological sample processing workflow.[1,2] As such, sample preparation methods have become a limiting factor to the advancement of downstream analytical techniques.[3] Many of the traditional methods used for sample preparation are time-consuming due to the multitude of steps needed. These steps can include substrate binding and several washes, liquid transfers, or dilutions. The time-intensive nature of these steps can result in sample loss and degradation.[2]

The utility of exploiting functionalized paramagnetic particles (PMPs) for analyte isolation has proven useful on a wide range of platforms.[4] One advantage of using PMPs is the ability to simply and thoroughly interrogate a fluid for analyte capture. In contrast with immobile functionalized surfaces, the PMPs can be suspended in a solution, allowing the functional surfaces of the PMPs to interact with a large portion of the fluid, without the need for complex mixing or flow focusing techniques. Another advantage is that the particles can be used in many different embodiments, as only a magnet is required for actuation and analyte isolation.[4–11]

The ways to isolate an analyte of interest from a given sample using PMPs can be further divided into three basic methods (Figure 1). In the first method, most commonly used in commercially available kits, background sample and any contaminants are removed by washing fluid (i.e., buffers)
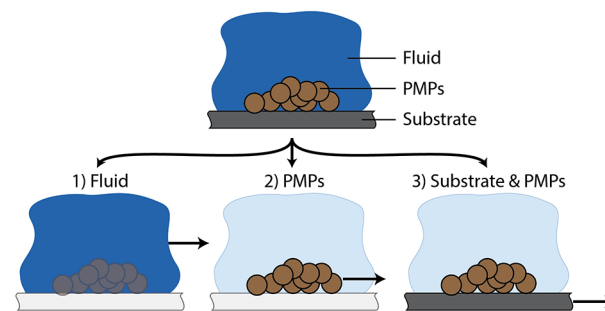

Figure 1. Different methods to isolate paramagnetic particles (PMPs) from a fluid. (A) Move the fluid: by keeping the PMPs and substrate stationary, fluid can be washed over the PMPs, isolating and purifying the analyte. (B) Move the PMPs: by moving the PMPs and binding analyte, the analyte can be effectively removed from an original sample into an elution buffer. (C) Move the substrate with the PMPs: by keeping the PMPs stationary with respect to the substrate, the substrate can be removed from the original sample and placed into an elution buffer.

over the substrate and immobilized PMPs.[4] Limitations of this widely used method include (1) the loss or dilution of the

original input sample, which limits the ability to reinterrogate the sample, (2) a time and fluid handling-intensive assay protocol due to the repeated liquid transfers required to wash the PMP-captured analyte,[1] and (3) loss of weakly bound analyte due to the time and shear stress required to perform an isolation.[5,12] In the second method, PMPs are selectively removed from a sample of interest. In these methods, analyte-bound PMPs are physically pulled from the original sample along the surface of a device, through an immiscible phase (e.g., oil), and into a second aqueous phase.[3,6,13−15] This method for PMP sample preparation has been highly effective at isolating analytes with high specificity and selectivity while simplifying workflows, and isolation can be performed in a matter of seconds.[6,7] Though effective, limitations for this method exist due to potential sample loss associated with the friction created by dragging the PMPs along a surface. Further, the need to incorporate oil[16] or surface[8]/geometric[5,15] pinning complicates these devices. In the third method of isolating PMPs, and the focus of this manuscript, analyte-bound PMPs are pinned to a surface, the surface is removed entirely from the background present in the original sample, and then the surface and PMPs are brought into contact with a second solution to elute the analyte. This method builds upon the benefits of simple workflows found with the second method but does not involve any dragging of the PMPs, significantly reducing loss due to friction and simplifying the device operation.

In this paper, we present a method that enhances the benefits of previous exclusion-based sample preparation methods and leverage a new technology called Sliding Lid for Immobilized Droplet Extractions (SLIDE). The SLIDE was developed to achieve gentle and reliable extraction of analyte-bound PMPs for sample preparation by leveraging surface tension of fluids and hydrophilic/hydrophobic patterning of surfaces. The operational principle of the SLIDE involves pulling analyte-bound PMPs to a hydrophobic surface (the "lid") and pulling the entire surface from an input sample to an output droplet. By creating simple hydrophilic pinning regions on the bottom plate, the surface tension of the fluid will maintain the droplets in place, while the PMPs can be moved from one droplet to the other without any loss due to PMPs dragging along the surface. Here, we demonstrate the ability of SLIDE to leverage simple fluid characteristics to create a robust and easy-to-use device for sample preparation for a range of analytes.

■ MATERIALS AND METHODS

SLIDE Lid and Base Fabrication. The SLIDE method uses two components, a base and a lid (Figure 2) that were rapid prototyped via stereolithography with Accura 60 (3D Systems, Rock Hill, SC). The base component serves as a holder for disposable cartridges, houses magnets located underneath the patterned fluid droplets, and acts as a guide for the handle. The lid contains two arms that guide the lid along the base and control the spacing between the bottom of the handle and the droplets. Built inside of the handle are vertical slots spaced 12 mm apart and designed to guide magnets during operation. Each of the magnets contained a stack of five 3/16 in. cube magnets (#B333-N52, K&J Magnetics, Jamison, PA), which were interconnected on the top by a thin steel bar (#36970754, MSC Industrial Supply Co., Melville, NY) to reduce the effect of the neighboring magnets by sharing a magnetic field. The distance between the handle bottom and the glass slide was 3 mm. While this distance was easily changed by altering the geometry of the SLIDE base or handle, operators should ensure
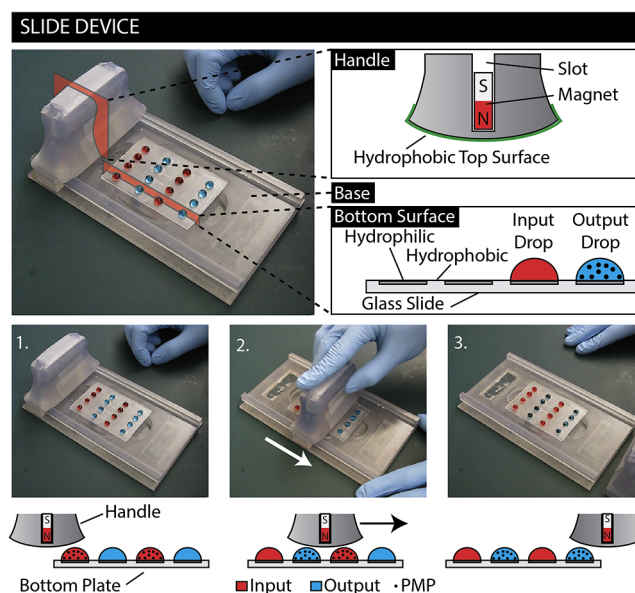

Figure 2. Image of the magnetic version of the SLIDE device. The device consists of a handle that houses the magnets and a hydrophobic layer, a base that houses the cartridge, and an insert that contains hydrophilic/hydrophobic patterns to position liquid drops (top right). SLIDE is simply operated by sliding the handle over the cartridge (bottom).

that the spacing is appropriate to establish contact between the SLIDE lid and the liquid droplets. For example, in preliminary experimentation with SLIDE (data not shown), we demonstrated successful SLIDE operation with droplet volumes ranging from 10 to 400 μL. As the droplet volume was changed, the spacing must be changed accordingly to establish contact between the droplet and lid.

SLIDE Operation. The SLIDE technology utilized surface tension and fluid droplet pinning dynamics for simple and low cost analyte isolation. A key advantage of the SLIDE was the usability of the device with an easily recognizable and intuitive platform that operates similar to that of a traditional credit card imprinter. (Younger readers may need to refer to http://www.youtube.com/watch?v=a7wutgAlNHk for a demonstration of how a credit card imprinter works.) As previously mentioned, the SLIDE operates by placing magnets on a top hydrophobic surface (the lid) and immobilizing PMPs relative to that surface. To redisperse the PMPs in the output fluid, the magnets must be removed from the lid, allowing PMPs to drop into the output fluid (Figure 2 and Supplemental Figure 1A, Supporting Information). Initially, this was accomplished using a system of cams that mechanically lifted the upper magnets as the lid passed over the output droplets of the SLIDE device (Supplemental Figure 1C, Supporting Information). However, we found that this cam system added unnecessary complexity to the system. In the final version of the SLIDE device, this mechanical actuation of the upper magnets was replaced with a magnetic field-driven mechanism. The upper magnets were allowed to freely move within vertical slots in the lid, while lower magnets of opposing polarity (1/4 in. magnetic disks, #D64-N52, K&J Magnetics, Jamison, PA) were held stationary in the base, below the cartridge. As the lid passed over the lower magnets, the upper magnets were repelled into the top of the lid. Because PMPs do not have permanent magnetism, they naturally repolarize and move toward the more powerful magnetic field. Thus, when the upper magnets were deflected

into the top of the lid, the PMPs traveled toward the lower magnet and dissociated from the lid surface (Supplemental Figure 1B, Supporting Information). During SLIDE operation, PMPs traveled to the lid surface when no magnet was present in the base (as in input droplets) and to the bottom surface when a magnet was placed in the base (as in output droplets).

SLIDE Operation Control. We performed a control experiment where the PMPs were drawn along a hydrophobic surface connecting the two droplets (as illustrated in the second method in Figure 1). Specifically, a hydrophobic surface (a Parafilm-coated glass slide) was mounted 3 mm above the surface of the SLIDE base such that the input and output droplets both made contact with this surface. The same cube magnets used in the SLIDE handle were used to draw the PMPs from the input droplet into the output droplet (with the hydrophobic surface held stationary). The quantities of PMPs that were successfully transferred were quantified via their autofluorescence in the red spectrum using a fluorometer (#Q32866, Life Technologies, Grand Island, NY).

SLIDE Disposables. The SLIDE integrates two main disposables, which were necessary for biological safety and cross-contamination minimization. The first was a cartridge patterned with wax to create a hydrophobic geometry to constrain the droplets. The substrate used for the cartridge was a 2 in. × 3 in. glass slide, and the wax was a paraffin-based wax (#D20-3, Sasol Wax, Hayward, CA). To pattern the wax on the slide, a stencil was made from silicone rubber (#31938707, MSC Industrial Supply Co.). The stencil consisted of a 4 × 4 array of 6 mm diameter holes separated by 19 mm and 12 mm in the length and width directions, respectively. This stencil was preheated on a hot plate at 105 °C, and melted wax was spread over the stencil with a transfer pipet. Next, a clean glass slide was placed on the stencil and allowed to sit until the wax covered the entire interface between the stencil and the glass slide. The stencil and glass slide were removed from the hot plate and allowed to cool at room temperature, at which point the silicone stencil was removed from the glass slide, exposing a (wax) hydrophobic region surrounding (glass) hydrophilic regions designed to hold 40 μL. The second disposable was a strip of parafilm (#P7793, Sigma-Aldrich, St. Louis, MO) that served as a hydrophobic barrier separating the droplet from the lid. The parafilm was held in place with Scotch tape (504662, Staples Inc., Framingham, MA) and changed between every operation. A step-by-step description of the process is illustrated in the Supporting Information (Supplemental Figure 2). The resulting thickness of the wax layer was approximately 0.1 mm.

Carryover Study. To assess the amount of fluid carryover in the SLIDE device, an acridine orange solution was made at a concentration of 0.5 mg/mL in stock solutions of 0%, 0.1%, and 1% Triton X-100 in DI water. For each experiment, 2 μL of Magnesil PMPs (#MD1471, Promega, Madison, WI) was added to each input solution of 40 μL. Droplets of deionized water were used as the output droplet. To evaluate the amount of carryover, an acridine orange dilution curve was created, and a linear fit was used to calculate the percent carryover based on the arbitrary intensity units measured using a Qubit Fluorometer with an excitation wavelength of 430−495 nm (Life Technologies).

Protein Readouts. To evaluate the utility of the SLIDE for protein purification, green fluorescent protein (GFP) was purified from a mixture of *E. coli* expressing both GFP and red fluorescent protein (RFP). Specifically, a solution containing 12.5 mg/mL of Protein G-conjugated PMPs (3 μm diameter, Dynabeads Protein G, Invitrogen) and 0.031 mg/mL anti-GFP antibody in PBS supplemented with 0.01% Tween 20 was prepared and incubated for 30 min at room temperature to allow antibody attachment to the PMPs. Following washing with PBS supplemented with 0.01% Tween 20, the antibody-labeled PMPs were resuspended in PBS (15 mg/mL PMP concentration) and 2% (by volume) *E. coli* bacterial lysate was added. At this dilution, the concentrations of the GFP and RFP were approximately 12 and 240 mg/mL, respectively. After incubating the GFP and RFP lysate with antibody-PMPs on a shaker for 10 min at room temperature, 50 μL of this solution was purified using SLIDE as previously described. The green and red fluorescence of the output droplets (and the input droplets) were measured with a fluorescent scanner (Typhoon Trio, GE Healthcare) to determine recovery and specificity, respectively. Samples were also run on an SDS-PAGE gel (NuPAGE 4−12% Bis-Tris Gel, Invitrogen) and silver stained (SilverQuest Silver Staining Kit, Invitrogen) to determine if GFP was effectively separated from the bulk of the non-fluorescent bacterial proteins.

DNA Readouts. Samples to measure DNA extraction were prepared by lysing LNCaP cells in Buffer RLT (Qiagen) for 5 min at room temperature with 2 μL of MagneSil PMPs (Promega). Lysates were prepared at concentrations of 1000 and 10 000 cells per 50 μL device input volume. Lysates were loaded onto SLIDE and processed as previously described. DNA was eluted from the PMPs in nuclease free water. As a comparison, other aliquots of this sample were purified using a conventional technique, where PMPs were captured against the side of a 1.5 mL microcentrifuge tube, the supernatant was removed, and the PMPs were resuspended in buffer (Promega Wizard Kit Wash Buffer). In the comparison samples, this wash process was repeated four times. Extracted DNA was amplified and quantified using qPCR on a LightCycler 480 (Roche) thermal cycler. Isolated DNA was mixed with 2× Taqman Gene Expression Master Mix (Life Technologies) and a commercially available assay for GAPDH genomic DNA (#4331182, Life Technologies). The thermal cycler ran 40 cycles of 60 °C for 1 min and 95 °C for 15 s, and threshold cycles (C_T) were calculated by the LightCycler software using the second derivative algorithm.

RNA Readouts. Samples to measure viral RNA extraction were prepared by spiking HIV viral-like particles (VLPs; viral envelope removed to render particles noninfectious; generous gift of Dr. Nathan Scherer) into fetal bovine serum (Gibco). Samples were lysed for 5 min at room temperature in Buffer MFL (Qiagen) in the presence of 2 μL of MagAttract PMP solution (2.8um diameter PMPs, Qiagen). Samples were prepared at VLP concentrations of 100 and 10 000 copies per SLIDE input volume. Samples were purified using SLIDE as previously described, and viral RNA was eluted into Buffer MFE (Qiagen). Reverse transcription was performed in a Techne TC-412 thermal cycler at 37 °C for 1 h followed by 85 °C for 5 min. The resulting cDNA was mixed with qPCR master mix (Taqman Gene Expression Master Mix, Life Technologies) and primers and probe specific to the LTR region of HIV (forward primer: 5′-GCCTCAATAAAGCTT-GCC-3′; reverse primer: 5′-GGCGCCACTGCTAGAGA-TTTT-3′; probe: 5′-AAGTAGTGTGTGCCC-3′; taken from Veronique et al. and synthesized by Life Technologies[17]). qPCR thermal cycling and analysis was performed as previously described for the DNA samples.

Cell Readouts. Prostate cancer epithelial cells (LNCaPs; ATCC) were cultured in RPMI 1640 media with 10% fetal bovine serum and 1% penicillin and streptomycin. Cells were suspended in media using treatment with trypsin and EDTA following conventional cell passaging protocols. Cells were stained green using Calcein AM (1:500 for 30 min at 37 °C), and 1000 cells were spiked into peripheral blood mononuclear cells (PBMCs) obtained by separating whole blood via centrifugation on a Ficoll gradient (GE Healthcare). For visualization, the PBMCs were stained red using CellTracker Red following the manufacturer's protocol. Streptavidin-coated PMPs (Dynabeads M-280) were coated with biotinylated anti-EpCAM antibody (Abcam product ab79079; 1 μg of antibody per mg of PMPs) via incubation for 15 min with tumbling at room temperature followed by washing with PBS with 0.01% Tween 20. Antibody-coated PMPs were mixed with cells and incubated for 30 min at room temperature with tumbling (50 μg of antibody-coated PMPs per sample). The cell/PMP mixture was loaded onto the SLIDE device as previously described, except that an intermediate droplet (positioned between the input and output droplet) was added. This droplet contained fluorescently labeled anti-EpCAM antibody (Abcam, ab112067). During operation, the SLIDE handle was moved from the input to the intermediate droplet and allowed to incubate for 15 min at room temperature to promote staining of the cells (the PMP/cell aggregate was not released into solution and remained as a flattened aggregate on the handle surface). After staining, the PMP/cell aggregate was released into the output droplet as previously described. The released cells were imaged using an epi-fluorescent microscope (IX-70, Olympus).

■ RESULTS AND DISCUSSION

Droplets Rolling on the Surface of the SLIDE. When a droplet of fluid moves along an inclined hydrophobic surface, it rolls on that surface, leaving behind little to no residue.[18−21] The flow profiles within the droplet reflect a fluid recirculation in droplets that are rolling down an inclined hydrophobic surface.[22] The interaction of the pinned fluid droplet within the SLIDE on the top hydrophobic surface was similar to these cases of a droplet rolling on inclined surfaces. The recirculation effect of a rolling droplet was experimentally validated in the case of the SLIDE (Figure 3A), demonstrating similarities in the two fluid systems. Because the fluid was rolling and not sliding down the surface, an important implication was the active detachment of the fluid from the receding edge of the fluid droplet that was caused by the surface tension of the fluid pulling away from the surface. This detachment ensured that there was a low amount of residual fluid left on the surface to contaminate the subsequent elution droplet. However, as a surface is separated from being in contact with a droplet, there will be residual fluid left on the surface or "carryover" from the fluid droplet. This concept of "carryover" was very important for the operation of sample preparation devices, as there can be many contaminants in an input sample that could interfere with downstream molecular analyses, and thus represents a critical area of study with the SLIDE device. Because of the similarities of fluid motion in the SLIDE to a droplet on an inclined surface, existing literature can be leveraged to better understand characteristics of the top surface that will result in lower carryover.

Recovery of PMPs. In a control experiment, PMPs functionalized to capture each analyte (protein, RNA, and
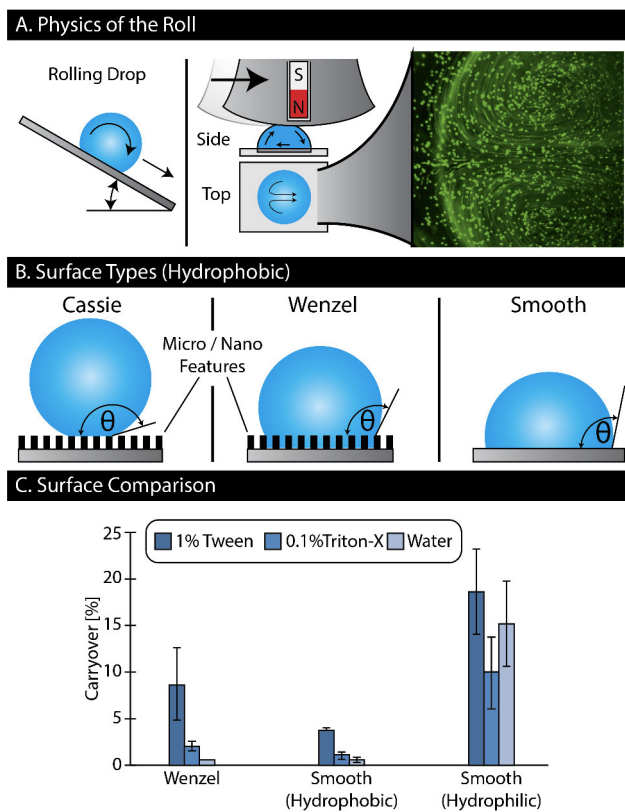

Figure 3. Churning effect of the fluid droplet. (A) Drop rolling on an inclined surface is similar to that found in the SLIDE (right). SLIDE image taken by suspending 1 μm of FITC microspheres into the fluid drop and opening exposure on the microscope for one second to show fluid motion. (B) Three types of hydrophobic surfaces shown. The Cassie surface has features in close enough proximity to prohibit wetting between the features, whereas the Wenzel surface has features far enough apart to permit wetting between. The smooth surface does not have these features. (C) The Wenzel and smooth surfaces were characterized using water and solutions of Tween in PBS. Both hydrophilic and hydrophobic smooth surface were tested for comparison.

DNA) were drawn from the input droplet to the output droplet along a stationary hydrophobic surface. While no measurable loss was observed with the DNA and RNA PMPs, we discovered that 20% of the protein PMPs (standard deviation of 3%, $n = 3$) were lost during transfer. It appeared that this loss was caused by the frictional forces encountered when "dragging" these PMPs across the stationary surface. In contrast, no measurable loss was seen with any of the PMP types when using the SLIDE mechanism. This result highlights a potential advantage of the "moving surfaces" strategy over the "moving PMPs" strategy (see Figure 1 for more illustrations of these strategies), particularly for certain PMP types.

Surface Properties and the Effect on Carryover. Surfaces can play a large role in the amount of carryover. Hydrophilic upper surfaces caused most biologically relevant fluids to "streak" along the top surface, resulting in high amounts of carryover. Hydrophobic surfaces minimized the interaction of the sample fluids to the surface and as such were exclusively explored for use with the SLIDE as the lid material. There are three classifications of hydrophobic surfaces that relate the "stickiness" of that surface to a fluid: Cassie, Wenzel, and smooth surfaces[21] (Figure 3B). In the example of a Cassie surface (a super hydrophobic surface), micro- and nanofeatures
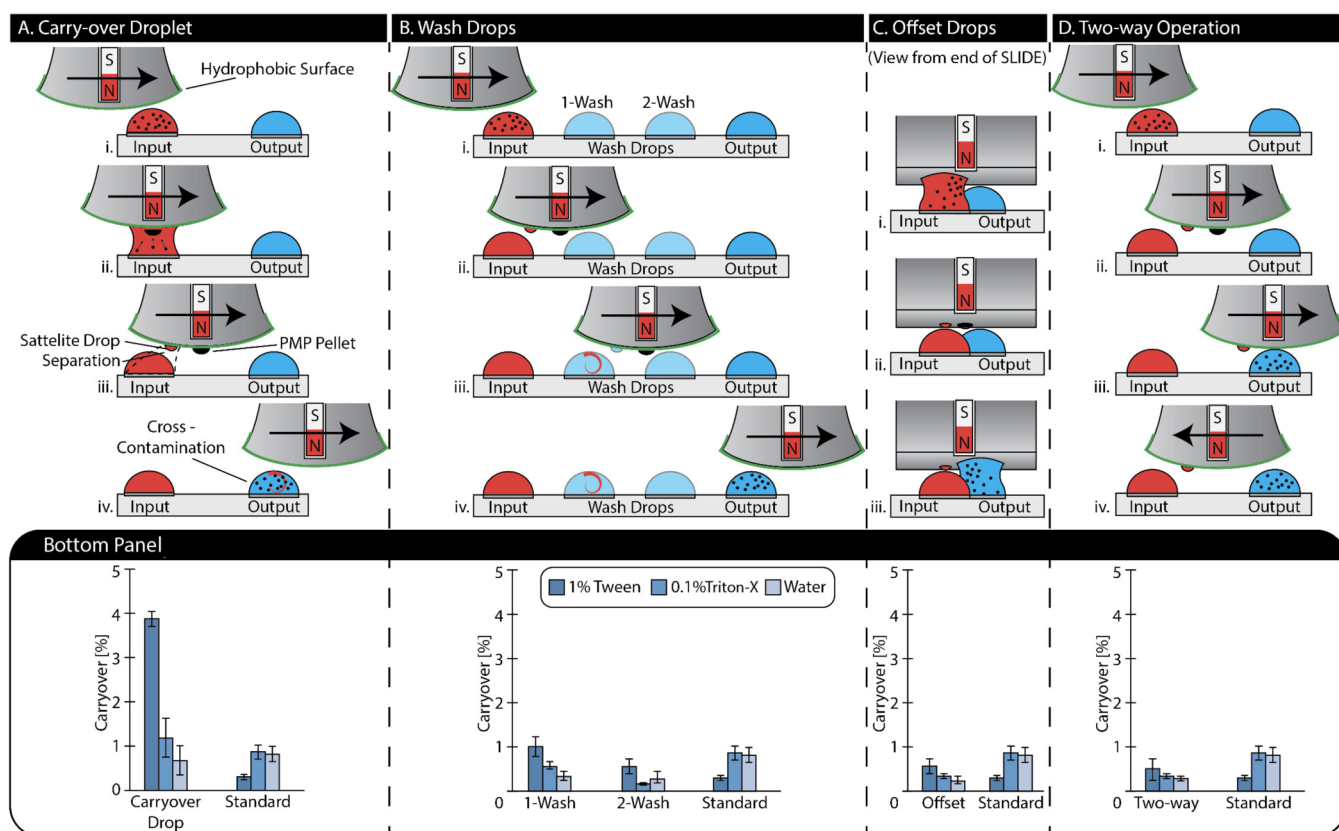
Figure 4. Generation of and mitigation of carryover. (A) A schematic demonstrates how a carryover droplet is created as the handle is removed from a drop. (B) Using wash drops can reduce the effect of carryover by rinsing and diluting. The wash method reduces the amount of carryover proportionally to the number of wash steps. (C) Offset drops are able to create a PMP pellet that is not in line with the carryover drop, thus allowing PMPs to be transferred without the carryover drop. (D) Two-way operation can be used to prevent carryover from ever contacting the output drop by moving the top surface backward before the carryover drop makes contact. Bottom Panel: Each method was characterized and compared to a standard macroscale technique. The data for each method is demonstrated below its respective method. In each case, error bars represent standard deviation of the mean ($n = 4$).

are used to create a thin layer of air below the sample droplet. While the hydrophobicity would be beneficial and potentially provide lower carryover, PMPs drawn by the magnetic force would be pulled into these spaces and get stuck, making deposition into the subsequent droplet challenging. Similar to a Cassie surface, the Wenzel surface relies on micro- and nanofeatures to improve surface hydrophobicity; however, unlike the Cassie surface, there is no layer of air, and the fluid contacts the surface directly. Though Wenzel surfaces had a higher contact angle than smooth hydrophobic surfaces, the "stickiness" of Wenzel surfaces resulted in more fluid and contaminants left behind, as this roughened surface carried more input fluid into the output fluid. This was experimentally validated by observing the carryover resulting from a hydrophilic top surface (cellulose acetate), a hydrophobic top surface (paraffin), and a Wenzel hydrophobic top surface (roughened paraffin). The smoother hydrophobic top surface was demonstrated to have the lowest carryover (Figure 3C).

Fluid Properties and the Effect on Carryover. While the composition of lid surface had significant impact on sample carryover, the physical shape of the lid was demonstrated to be important. In order to create a device with simple user operation (similar to a credit card imprinter), the SLIDE was designed with a lid that the user could move across the sample in a single motion to perform all purification steps and have immediate access to the purified analyte. This operation

required the lid to contact a fluid droplet and then be removed from that fluid droplet. However, when the edge of a lid passed over a fluid droplet, contact was severed between the fluid and the lid. This breakup event often resulted in the deposition of a residual "satellite" droplet on the surface of the lid (Figure 4A). During SLIDE operation, this satellite droplet was composed of original sample material and occasionally resulted in excess carryover. The influence of fluid properties was tested to evaluate the impact on the amount of material carried within this satellite droplet. Fluid viscosity did not seem to impact the amount of carryover to a viscosity of approximately 10 cP. However, fluid viscosities in excess of 10 cP yielded a high Stokes' drag, preventing the PMPs from moving toward the magnet (Supplementary Figure 3, Supporting Information). In these cases, the droplet acted as a ferrofluid and followed the magnet out of the hydrophilic pinning region. Surface tension was found to affect the size of the satellite droplet, as increasing the amounts of Triton-X 100 (i.e decreasing surface tension) in the solution resulted in increased contaminant carryover (Figure 4A).

SLIDE Design to Mitigate Carryovers. In order to mitigate the effect of satellite droplets, a curved lid was designed to promote a controlled and reproducible droplet, while maintaining operational simplicity. When using a flat lid, the droplet dissociated at the edge quickly, yielding a large and variable satellite droplet. The curved lid reduced the size of the
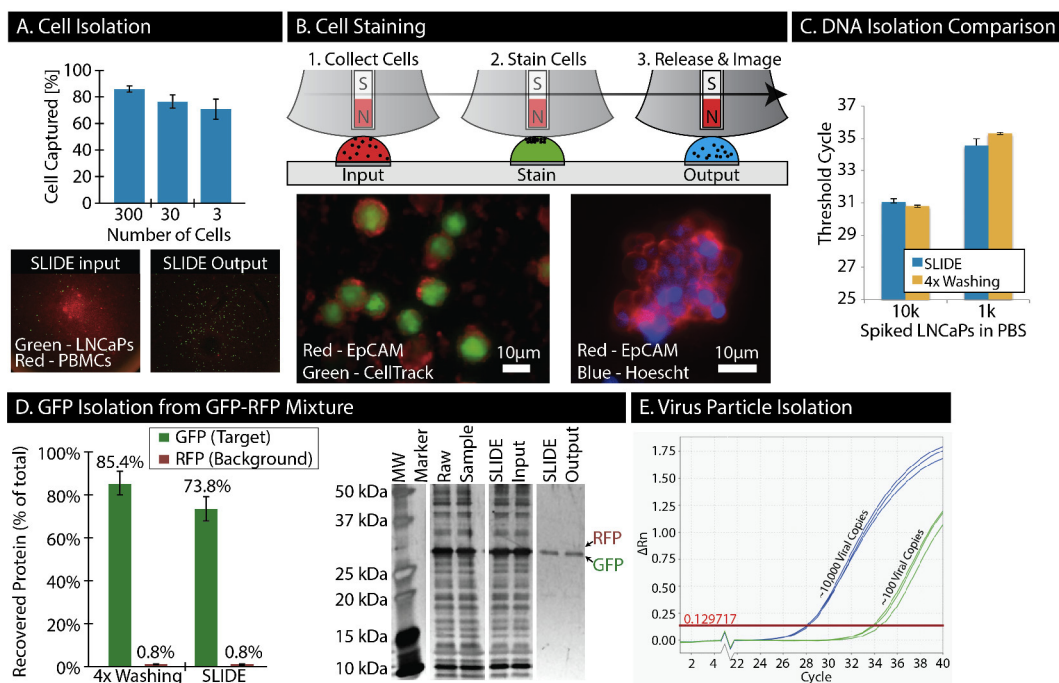
Figure 5. Applications of the SLIDE. (A) The SLIDE can be used for cell capture from a background of 5 million fixed peripheral blood mononuclear cells with an efficiency of >70% for each of three cellular densities (300, 30, and 3 cells, $n = 3$ per experiment). The purification efficiency was high, with $99.997 \pm 0.0078\%$ of nontarget cells left behind in the first well (an average of 134 ± 39 nontarget cells remaining, $n = 3$). (B) Cell staining using SLIDE involves loading the samples, moving the lid from being positioned over the input drop to collect sample, to over a staining drop to perform cell staining methods, to a final release well to image the cells. Cells in the left panel were stained with cell tracker and EpCAM surface antibodies, and cells in the right panel were stained for Hoescht and EpCAM. (C) DNA isolation using SLIDE shows comparable DNA extraction from lysed LNCaPs to standard washing methods. (D) Low carryover of the SLIDE demonstrated with GFP isolation from complex GFP-RFP expressing *E. coli* bacterial lysates, with an efficiency of >70% and a specificity of GFP capture of >99% compared to RFP nonspecific carryover ($n = 3$, left). Silver stained gels demonstrate the purity of the sample from background proteins (right). (E) HIV virus-like particle isolation demonstrated in the SLIDE from lysed human plasma spiked samples.

satellite droplet by gradually removing the top surface from the fluid droplet, while maintaining the one-direction swipe operation of the device. However, due to the nature of fluid separation from a surface, a small droplet ($\sim$0.1 μL) was still left behind, the size of which differed with various fluids and operating parameters. In most conditions tested, the amount of carryover from the input droplet to the output droplet using a Parafilm surface was below 2%. If there is a concentration step (e.g., the output droplet is smaller than the input droplet), however, this percentage could be higher. There are many solutions that could be implemented to mitigate this carryover. Three methods for decreasing carryover were evaluated for their efficacy and operational simplicity: (1) wash droplets were placed between the input and output droplet (Figure 4B), (2) the center of the input droplet was offset from the magnet and output droplet (Figure 4C), and (3) a two-way operation of the lid where the PMPs were taken to the output droplet, and then the lid was pulled backwards before the satellite droplet could contact the output droplet (Figure 4D).

Wash Droplets. Droplets of fluid placed between the input and output droplets that came in contact with the SLIDE lid surface allowed contaminants to reconstitute into the intermediate buffers instead of into the output droplet. Assuming that the main source of contamination in the SLIDE was due to the carryover instead of interstitial space of the collected PMPs,[6] the PMPs did not need to be mixed into each of the wash steps to effectively remove the source of contamination. To test this method of purification, three sets of devices were tested: (1) no wash droplet, (2) one wash droplet,

and (3) two wash droplets. Each wash was seen to produce a 2-fold removal of background contaminant, with the contamination in the two-wash device in all cases to be below 0.6% (Figure 4B).

Offset Droplets. As the lid was removed from the droplet, the highest point of the drop was naturally above the geometric center of the pinning region. This represents the point at which the drop detaches and forms a satellite drop on the upper surface. Because the droplets used were semispherical in shape and the magnet is above the center of the droplet, the satellite droplet and PMP pellet are both aligned with the apex of the droplet. However, by offsetting the input droplet relative to the magnet or by changing the geometry of the input droplet, the apex could be guided away from the PMP pellet as the lid was moved out of the input droplet. By separating the apex from the PMP pellet, the satellite droplet was no longer collinear with the output droplet and the PMP pellet. As a result of this simple geometrical change, the satellite droplet avoided the output droplet, preventing contact and thus carryover of input fluid via this satellite droplet (Figure 4C). When this hypothesis was tested, it resulted in carryover below 0.6% (Figure 4C).

Two-Way Operation. The SLIDE was designed to work akin to a credit card imprinter, in that one swipe produced purified, isolated samples. However, to avoid introducing the carryover droplet to the elution droplet, a two-way operation was introduced to only allow the PMPs to drop into the elution and pull the lid back toward the input droplet prior to introducing the satellite droplet to the output. The observed carryover with this method was below 0.6% (Figure 4D).

These methods for reducing carryover were chosen for their user friendliness when operating the SLIDE while reducing the sample carryover. Specifically, (1) the wash droplets were placed in line with the SLIDE, resulting in no change of the operation of the device. (2) Offset droplets caused the carryover droplet to be diverted in a way that is invisible to the operator, again resulting in no change of the operation of the device. For the two-way operation (3), a jam is simply placed on the device such that carry over drops never reached the output drop and following isolation the handle was slid back to its starting position.

Applications of the SLIDE Isolation Technique. To demonstrate the utility of the SLIDE to isolate a variety of analytes, cell, protein, and DNA captures were performed in the SLIDE device. A specific cell type was isolated with high specificity (>90%, Figure 5A) from a heterogeneous mixture of multiple cells (similar to the buffy-coat layer after a Ficoll-Paque density centrifugation of blood samples). Leveraging the in-droplet mixing that occurs during the operation of the SLIDE, a simplified staining protocol was performed. Cells remained on the lid during staining and were transferred into a PBS containing output droplet for imaging. In less than an hour and within a single linear operational path, cells were bound to PMPs, isolated from background cells, stained, and placed into a droplet for imaging (Figure 5B). To demonstrate the purity and specificity of analyte isolation from complex samples using the SLIDE, we evaluated the ability to isolate GFP from a complex cell lysate containing GFP, RFP, and cellular proteins. Without performing any additional washing steps beyond the previously described SLIDE protocol, we demonstrated highly specific capture of GFP using a silver stained gel (Figure 5D). This result demonstrates that SLIDE can specifically isolate a protein of interest without substantial nonspecific capture. On the basis of the results of the carryover quantification experiments (Figure 3C), we decided to modify our nucleic acid protocols to include a wash volume between the sample and elution buffers. Specifically, Wizard Wash Buffer (Promega) was used for the DNA samples and Buffer MFW2 (Qiagen) was used for the viral RNA samples. qPCR of the DNA indicated that there was no significant difference in C_T values between samples purified with SLIDE and those purified with conventional washing methods (Figure 5C). Furthermore, the isolation of viral RNA using SLIDE demonstrated the high precision of this method, including SLIDE's ability to handle samples with low numbers of analyte copies (~100, Figure 5E). Taken together, these data demonstrate that SLIDE is a viable alternative as a sample preparation process for isolating nucleic acids.

CONCLUSIONS

We have presented a sample preparation method that leverages moving the substrate instead of the fluid containing sample. While improving the operational simplicity of sample preparation, the SLIDE also eliminates the need for dilutive and/or harsh washing steps during which analyte can be lost,[5,12,23] the need for immiscible fluids, or complicated manifolds for collecting and purifying analyte from a complex sample. The SLIDE is enabling for the simplicity and inherent usability of the device; operation of the SLIDE is similar to a credit card imprinter and needs few parts to operate. During initial characterization experiments, a mode of device failure was found when using low surface tension samples, resulting in nonspecific carryover of sample into the elution buffer. It was

demonstrated that this carryover was a result of satellite droplets created when the fluid detaches from the SLIDE lid. Three strategies to mitigate the effect of this satellite droplet were evaluated, including (1) adding nondilutive wash droplets between the input and output droplets, (2) changing operation to a two-step motion, and (3) offsetting the input droplet from the output droplet. Without contamination mitigation strategies, approximately 2% of the sample (from a 40 μL sample) was carried over into the output droplet. While this may be adequate for certain downstream analysis techniques, a carryover of approximately 0.6% was achieved when using any of the three strategies, putting SLIDE performance on par or better than existing commercial purification protocols. These methods for the SLIDE maintain a simple operational workflow for analyte purification that is both robust and precise. Further, the simple workflow can be translated to cell capture and staining protocols to streamline these processes, saving time and reagents. The SLIDE is an enabling method, as the fundamental operational principles will translate to simple embodiments that can be employed for the high purity isolation of any analyte.

ASSOCIATED CONTENT

S Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: scott.michael.berry@gmail.com.

Author Contributions

‡B.P.C. and D.J.G. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): All authors hold equity in Salus Discovery LLC, which has licensed some of the technology described in the manuscript.

ACKNOWLEDGMENTS

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