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The K-Channel: A Multi-Functional Architecture for Dynamically Re-Configurable Sample Processing in Droplet Microfluidics

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Abstract

By rapidly creating libraries of thousands of unique, miniaturized reactors, droplet microfluidics provides a powerful method for automating high-throughput chemical analysis. In order to engineer in-droplet assays, microfluidic devices must add reagents into droplets, remove fluid from droplets, and perform other necessary operations, each typically provided by a unique, specialized geometry. Unfortunately, modifying device performance or changing operations usually requires re-engineering the device among these specialized geometries, a time-consuming and costly process when optimizing in-droplet assays. To address this challenge in implementing droplet chemistry, we have developed the “K-channel,” which couples a cross-channel flow to the segmented droplet flow to enable a range of operations on passing droplets. K-channels perform reagent injection (0–100% of droplet volume), fluid extraction (0–50% of droplet volume), and droplet splitting (1:1–1:5 daughter droplet ratio). Instead of modifying device dimensions or channel configuration, adjusting external conditions, such as applied pressure and electric field, selects the K-channel process and tunes its magnitude. Finally, interfacing a device-embedded magnet allows selective capture of 96% of droplet-encapsulated superparamagnetic beads during 1:1 droplet splitting events at ~400 Hz. Addition of a second K-channel for injection (after the droplet splitting K-channel) enables integrated washing of magnetic beads within rapidly-moving droplets. Ultimately, the K-channel provides an exciting opportunity to perform many useful droplet operations across a range of magnitudes without requiring architectural modifications. Therefore, we envision the K-channel as a versatile, easy to use microfluidic component enabling diverse, in-droplet (bio)chemical manipulations.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Videos of K-channel injection; K-channel extraction; K-channel stable interface; K-channel droplet splitting; injection with pulsed electric field; serial K-channel operation; K-channel material exchange; K-channel-enabled enzyme chemistry; K-channel magnetic bead capture; magnetic bead detection; and magnetic bead washing. Micrograph of K-channel oil extraction. Plots and micrographs of the directional independence of K-channel operations; K-channel cross-channel angle characterization; and magnetic bead quantitation.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

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Graphical Abstract

In contrast to bulk approaches or continuous flow microfluidics, droplet microfluidic systems compartmentalize samples and reagents into nano- to femtoliter-sized reactors in an immiscible oil.^{1–3} By segmenting flow into discrete plugs separate from each other and from device walls, these systems minimize sample loss from adsorption to channels or tubing, and enable generation of libraries containing thousands of individually addressable reaction volumes.^{4–6} Capitalizing on rapid mass and heat transfer in droplets, droplet microfluidic devices have been applied to synthesis and nanotechnology.^{7,8} Furthermore, droplet dimensions are especially suitable for analysis of small biological samples or even single cells, and adjusting operation times to handle variable numbers of droplets accommodates a range of sample volumes.^{9–11} Notable recent work leverages droplet microfluidic systems as miniaturized instruments for immunoassays,^{12,13} epigenetic analysis,¹⁴ and directed molecular evolution,¹⁵ among other applications.

In order to achieve complex biochemical processing in droplets, devices must demonstrate a number of capabilities. Droplet or plug formation often occurs at T-junction and flow focusing structures.^{16,17} Picoinjection^{18–20} or droplet fusion²¹ add reagents into sample droplets. Droplet reactions incubate in delay channels²² or off-device followed by reinjection.²³ Droplet splitting samples from or parallelizes reactions.^{24–26} Coupled with antibody-functionalized magnetic beads, droplet splitting can even selectively concentrate samples and remove waste volume.^{13,27–31} Overall, these well-characterized approaches provide a toolkit for in-droplet chemistry, and recent reviews highlight the broad range of droplet techniques and applications.^{7,8,17,32,33}

Realizing the potential of droplet microfluidics will require overcoming certain limitations. For instance, many droplet operations occur in highly specialized geometries, and optimizing performance for a particular chemical system (such as changing the volume of fluid injected or removed) may necessitate fabricating a device with modified dimensions. Soft lithography enables rapid prototyping, but the multiple device iterations needed to optimize an in-droplet assay can still lead to high time and monetary costs.³⁴ An additional challenge includes the sensitivity of droplet manipulations like injection to flow instabilities and contamination. Because these techniques often rely on a single inlet channel for injected reagent delivery, pressure fluctuations during extended device operation significantly move the equilibrium position of the injection interface (changing injection magnitude), and any

material extracted from droplets during injection may contaminate later droplets.^{19,35,36} Finally, common biochemical techniques like ELISA typically require washing samples on a solid phase like antibody-conjugated magnetic beads,³⁷ but only limited efforts have demonstrated analogous processes in continuously flowing droplets at low throughput (1–5 droplets per second) compared to many other droplet operations (100–1000 droplets per second).^{38,39} Therefore, the clear need exists to engineer versatile and robust droplet manipulation modules that simplify assay optimization and enable in-droplet washing.

With these considerations in mind, a promising approach to creating more robust droplet systems with useful, novel functions has been “cross-channel”-style geometries. As a pioneering first example, Chen et al. developed the “chemistode,” a probe for delivering nL droplets via one channel and collecting sample droplets with the second across the parallel surface being interrogated.⁴⁰ Later examples have integrated miniaturized cross-channel architectures into fully self-contained droplet systems, typically as a means of droplet formation. Lin, et al. used a cross-channel flow of a cell suspension past an oil channel to generate single cell-containing droplets-on-demand (DoD) upon nearby PDMS valve triggering.⁴¹ This structure stably maintained the aqueous cell suspension-oil interface before and after valve actuation. More recently, a similar approach adapted a cross-channel structure which dispersed flow instabilities through the cross-channel to improve droplet formation reproducibility.³⁶ A serial array of similar modules also served as a combinatorial droplet generator for DNA library assembly.^{6,42} Other variations on cross-channel flow interfaced to droplets have been specialized to single operations.^{38,43}

Aiming to develop a simple, easily switchable module for droplet manipulation, we have explored cross-channel interfaces that support droplet generation and sampling modes beyond those previously reported.^{6,36,40–43} By adding features such as on-chip electric and magnetic fields, our “K-channel” device enables a range of useful droplet processing steps such as reagent injection, volume extraction, droplet splitting, magnetic bead capture, and more. Importantly, our approach allows dynamic tuning of a single device among these different functional operations simply by adjusting external device operating conditions, such as applied pressure, electric field, and fluid composition. Moreover, continuous cross-channel flow greatly reduces the chance for cross-contamination inherent in previous approaches.^{19,35} Finally, we demonstrate the versatility of the K-channel module to create a two-step washing device consisting of magnetic bead-concentrating droplet splitting followed by water injection, and realize robust device operation at nearly two orders of magnitude higher droplet frequency than previous reports.^{38,39}

EXPERIMENTAL SECTION

Microfluidic Device Fabrication

PDMS microfluidic devices bonded to a glass substrate were formed via conventional soft lithography using SU8-2025 Photoresist (MicroChem Corp.) masters on 3” silicon wafers (WRS Materials).³⁴ Photomasks were designed in AutoCAD (Autodesk) and printed as transparencies (CAD/Art Services). Prior to use, masters were treated with (tridecafluoro-1,1,2,2-tetrahydrooctyl)tri-chlorosilane (Gelest, Inc.) under vacuum for 2–4 hours. PDMS was mixed 10:1 (RTV615A:RTV615B) (Momentive) and cured for 1 hour at

70 °C. PDMS molds were removed from the master and punched with 20 Ga needles to create ports. PDMS molds and glass slides (Cover Glass, Thickness 1½, 22 × 40 mm, Corning, Inc.) were bonded following oxygen plasma activation (PDC-32G, Harrick Plasma). Devices were incubated at 70 °C overnight and treated with 1% (tridecafluoro-1,1,2,2-tetrahydrooctyl)trichlorosilane in FC-40 (Sigma Aldrich). All channels were 40 µm in height.

Fluid Flow

Fluids were driven by a home-built pressure controller. Briefly, a custom gas manifold (VWR International) distributed N₂ through a parallel regulator array (VWR International) into an array of LHDA0531115H solenoid valves (The Lee Company). Valves were actuated by a NI PCIe-6251 Multifunction Data Acquisition device (National Instruments) interfaced via LabView (National Instruments). Upon valve actuation, pressure was delivered into the headspace of sample vials via stainless steel fittings (New England Small Tube). Fluid flowed from pressurized vials through #30 AWG PTFE tubing (Cole Parmer) inserted directly into device ports. Typical pressures were 65 kPa for the continuous phase, 60 kPa for the dispersed phase, and 20–90 kPa for the cross-channel fluid. Typical droplet volumes were 100–200 pL, and droplets were usually produced at 300–500 Hz.

For all experiments, the continuous phase was 3M Novec 7500 Engineered Fluid (The 3M Company) with 2% poly(ethylene glycol) di-(krytox-FSH amide) (Ran Biotechnologies, Inc.) surfactant. For geometry characterization (Figures 2, 3, S-1, S-2, and S-3 and Movies S-1, S-2, S-3, and S-4) and for serial K-channels (Movie S-6), the dispersed phase was food dye (McCormick & Company) in water, and the fluid in the K-channel was identical either to the continuous phase (for droplet splitting) or to the dispersed phase (for other operations). Injection with pulsed electric field (Movie S-5) used water as the dispersed phase and 1 mM fluorescein (Sigma Aldrich) in water as the K-channel fluid. For material exchange characterization (Figure 4 and Movie S-7), the dispersed phase was 200 µM fluorescein in water, and the fluid in the K-channel was water. For the enzyme characterization (Movie S-8), β-galactosidase (Life Technologies Corporation) converted resorufin β-D-galactopyranoside (ThermoFisher Scientific) into fluorescent resorufin in 1X phosphate buffered saline with 0.5% bovine serum albumin. Where indicated, an electric field (25–35 VAC) was supplied to 3 M NaCl in electrode channels²⁰ or to 250 µm Pt wires submerged in solution vials by a Tenma 72–6628 DC Regulated Power Supply (MCM Electronics) modified with a DC to AC inverter. Electric field pulses were actuated manually or by a LabView interface.

Magnetic Bead Capture and Washing

Embedded magnet devices were fabricated as described above with the addition of a magnet that was positioned along guide features on the master prior to pouring PDMS. The magnet was removed to form a guide hole in the PDMS mold after curing. Magnets were 1/2" × 1/4" × 1/8" N52 (magnetized through thickness) (K&J Magnetics, Inc.). Prior to device operation, an array of 8 magnets was positioned along the guide hole to form a block of 4 magnets above and 4 magnets below the glass slide (total dimensions 1/2" × 1/2" × 1/2").

The magnetization of this array was along the plane of the glass slide in the direction of the main channel side of the K-channel.

1–3 μm paramagnetic bead samples (Protein A Magnetic Beads, New England Biolabs) were washed with buffer (50 mM Trizma HCl at pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.5% IGEPAL CA-630, 1% Triton X-100), suspended in Optiprep Density Gradient Medium (Sigma Aldrich), and filtered through a microfluidic device with 10–15 μm pores to remove contaminating particles and large aggregates prior to use.

Droplet Imaging and Analysis

Microfluidic devices were imaged by an M80 stereo microscope (Leica Microsystems) or a DMi8 light microscope (Leica Microsystems) using a Phantom Miro eX2 high speed camera (Vision Research). Videos and images were typically collected at 1000–6000 fps. For geometry and material exchange characterization, droplet size and frequency were analyzed using Droplet Morphometry and Velocimetry (DMV) software, courtesy of Amar Basu (Wayne State University, Detroit, MI).⁴⁴ Fluorescent analysis was carried out using ImageJ (NIH) to monitor pixel intensity followed by processing by an in-house macro. All plots use $N = 3$ devices in which at least $N = 30$ droplets were measured on each device at each experimental condition.

Magnetic bead analysis was performed semi-manually in ImageJ to track droplet size and magnetic particles in droplets across the detection channel (see Figure S-4). Bead capture analysis (Figure 5) tracked 300 main channel and 300 waste channel droplets ($N = 100$ droplets for each of $N = 3$ devices) and bead washing analysis (Figure 6) tracked 180 droplets ($N = 20$ droplets for $N = 3$ time points for $N = 3$ operations: droplet formation, splitting, and injection) across multiple frames to reduce error from beads obscured by droplet edges and from aberrations due to extra-droplet satellites and channel defects.

RESULTS AND DISCUSSION

K-Channel Design

The K-channel is formed by the intersection of a continuous flow cross-channel with a segmented flow main channel (Figure 1). This junction resembles a capital letter “K” turned on its side, and also typically includes nearby electrode channels.²⁰ As droplets pass the intersection, the flow through the cross-channel predictably modifies them based on applied conditions. These operations include, but are not limited to, injecting fluid from the cross-channel into droplets, extracting fluid from droplets into the cross-channel, maintaining a stable interface without altering droplets, using oil (instead of aqueous) cross-channel flow to split droplets, and adding or extracting oil to change droplet spacing. For operations such as injection and extraction, droplets merge with the cross-channel flow temporarily during destabilization via a local electric field, and the continuously flowing cross-channel washes away extracted material to reduce the risk of droplet-to-droplet contamination. While approaches like picoinjection use a single inlet flow channel to add reagents,^{19,35} the K-channel inlet-outlet functions to partially disperse flow instabilities so that operations are less sensitive to instabilities arising during extended operation. As a consequence of its

versatility and robustness, K-channel devices and arrays of K-channels demonstrate significant potential for rational design and dynamic reconfiguration of complex droplet reaction sequences.

Fundamental K-Channel Operations

K-channel behavior with aqueous flow through the cross-channel follows the Hagen-Poiseuille Equation:⁴⁵

$$Q = \Delta P / R_H \quad (1)$$

where Q is the volumetric flow rate through a system volume, P is the pressure drop across the system volume, and R_H is the hydraulic resistance of the system volume derived from the viscosity, the channel length, and the channel cross-sectional area.

Selecting the junction of the cross-channel with the droplet channel as the system volume specifically describes the K-channel:

$$Q_J = \Delta P_J / R_{H,J} \quad (2)$$

where Q_J is the flow through the junction (from the cross-channel into the main channel), P_J is the pressure drop across the junction, and $R_{H,J}$ is the hydraulic resistance of the junction. $Q_J > 0$ corresponds to injection, $Q_J < 0$ corresponds to extraction, and $Q_J = 0$ corresponds to no net droplet volume change.

The pressures for droplet formation applied to the oil continuous phase, P_C , and to the aqueous dispersed phase, P_D , are held constant. Assuming constant hydraulic resistance for a single device at equilibrium, varying the pressure applied to the K-channel inlet, P_K , leads to:

$$\Delta P_J = c_1 * \Delta P_K + c_2 \quad (3)$$

where c_1 and c_2 are constants derived from P_C , P_D , and the hydraulic resistances of the device. Clearly, P_J is directly proportional to P_K . Substituting Equation 3 into Equation 2:

$$Q_J = c_3 * \Delta P_K + c_4 \quad (4)$$

where c_3 and c_4 are constants derived in the same manner as c_1 and c_2 . Thus, Q_J is also directly proportional to P_K . Because it alters the sign and magnitude of Q_J , changing P_K enables injecting into or extracting from droplets over a working range of volumes.

Removing the destabilizing electric field establishes a relatively high $R_{H,J}$ based on the Laplace pressure of the aqueous-oil interface.¹⁹ Under a limited range of pressure

conditions, this can be exploited to approximate $Q_J = 0$, where a robust droplet-K-channel fluid interface prevents volume exchange between droplets and the aqueous cross-channel flow. At slightly lower pressures (where $Q_J < 0$ and $|Q_J|$ is small) oil may stream out from the main channel into the K-channel outlet to decrease the spacing between droplets. Significant deviations from $Q_J = 0$ result in irregular droplet splitting (for $Q_J < 0$) or new droplet formation from the cross-channel fluid (for $Q_J > 0$). On the other hand, oil flow (instead of aqueous flow) through the cross-channel can lead to both dispersed and continuous phase removal through the K-channel (droplet splitting and oil removal) when $Q_J < 0$. For $Q_J > 0$ with oil flow, additional oil enters the main channel to increase the spacing between droplets.

Figure 2 displays the fundamental K-channel operations described by this model. Representing a typical device geometry, the 40 μm wide main channel (flowing left to right) intersects with the cross channel (flowing right to left) using a 25 μm wide inlet channel (right) and a 40 μm wide outlet channel (left). Droplets formed upstream at a T-Junction under constant pressure. While manipulating the pressure at the K-channel inlet, relatively high pressure (85 kPa) injected orange dye from the cross-channel into passing droplets in the presence of an electric field (Figure 2A and Movie S-1). Decreasing the pressure (45 kPa) extracted fluid from droplets into the cross-channel in the presence of an electric field (Figure 2B and Movie S-2). Moderate pressure (73 kPa) without electric field created a stable and robust droplet-cross-channel interface (Figure 2-C and Movie S-3). Finally, oil flow with low pressure (20 kPa) and without electric field yielded droplet splitting (Figure 2D and Movie S-4). As Figure 2 demonstrates, changing the applied conditions (pressure, electric field, fluid), enabled a wide range of operations, not only with a single geometry, but by a single device, a novel capability which will be useful in designing and optimizing modular droplet microfluidic assays.

Device Architecture Characterization

The performance of the K-channel over typical operating conditions is shown in Figure 3. All data was collected using a standard K-channel geometry (see Figure 2) unless otherwise noted. In Figure 3A, the net change in droplet volume due to interaction with the aqueous phase in the K-channel across a range of K-channel pressure is plotted. Depending on the applied pressure, up to 50% of droplet volume could be extracted and up to 100% of droplet volume could be injected. Additionally, in the absence of an applied electric field, a stable interface between droplets and the K-channel (in which droplet volume and composition were unaffected) was supported over a ~ 12 kPa range. At the lower pressures of this stable interface range, oil was selectively removed from the main channel, decreasing droplet spacing but leaving droplet composition and volume unchanged (see Figure S-1). While operating in this stable range, pulsed application of the electric field enabled injections into fractions of the total droplet population (Movie S-5). The number of droplets receiving injections was defined by the droplet frequency and the duration of the applied electric field. Importantly, injection and material transfer only occurred in the presence of the electric field, so droplets passing the K-channel before or after the electrical pulse were unaltered. This performance was robust across multiple replicates of each device geometry ($N = 3$).

Equation 4 predicts that fluid flow from the cross-channel into droplets should be directly proportional to the pressure applied to the K-channel inlet, but it is clear that net droplet volume change does not follow a linear trend (Figure 3A). Because droplet formation and the K-channel are controlled via applied pressure, changing the pressure at one inlet changes the relative pressure drop and, therefore, volumetric flow rate, across every channel in the device. Thus, droplet frequency and, to a lesser extent, droplet volume decrease as the K-channel pressure increases.¹⁶ By taking the product of the net change in volume per droplet, $V_{Net,Droplet}$ and the droplet frequency, f , at each pressure, we obtained approximate values for the volumetric flow rate from the cross-channel into droplets, Q_j :

$$Q_j = \Delta V_{Net,Droplet} * f \quad (5)$$

Plotting this flow rate versus K-channel pressure yielded a linear relationship ($R^2 = 0.981$) consistent with the model (Figure 3B). Minor deviations from linearity are not surprising given limitations of the single phase, laminar flow-based Hagen-Poiseuille-based model,⁴⁵ which also does not account for any oil extraction from the inter-droplet spacing during K-channel operation.

While changing pressure enabled a range of operations, K-channel behavior can also be tuned by varying the geometry. Most significantly, altering hydraulic resistances (by changing the K-channel inlet width across a range of sizes from 15 μm to 40 μm) adjusted performance (Figure 3C). As the width decreased, the channel hydraulic resistance increased, so a greater fraction of the pressure drop occurred across the length of the inlet channel instead of across the junction between the cross-channel and the main channel. Flow into droplets became more negative at each pressure. On the other hand, flow into droplets increased when the hydraulic resistance of the K-channel outlet increased (data not shown). For this reason, the standard K-channel geometry (25 μm inlet, 40 μm outlet) is asymmetrical: changing cross-channel flow direction (40 μm inlet, 25 μm outlet) selects between two ranges for K-channel pressures on a single device when desired. Nonetheless, flow direction does not intrinsically affect net droplet volume change. A symmetric K-channel with 40 μm wide inlet and outlet channels did not significantly differ in performance with flow direction at 95% confidence (Student's t-Test, Figure S-2). Likewise, changing the angular orientations of the cross-channels had little effect (Figure S-3) relative to changing channel widths. By altering the geometry, K-channels can be rationally designed to provide desired operations in a chosen pressure range.

The K-channel also provides a simple method for tunable droplet splitting. Adjusting the pressure on the K-channel altered droplet splitting so that 15–50% of the droplet (1:5 1:1 daughter droplet ratio) was diverted into the cross-channel in each splitting step (Figure 3D). This method provides an advantage over many previously described droplet splitting regimes: it does not require prototyping a new device with a different ratio of hydraulic resistances to adjust the size of daughter droplets,^{24,25} a capability not widely demonstrated by existing methods.^{26,28} At high K-channel pressure, oil was injected into the main channel to increase droplet spacing, but droplets did not split (data not shown).

Finally, K-channel devices are not limited to single operations. Instead, serial or parallel K-channels can enable multistep sequences on a single device. For example, two K-channels were operated in series (Movie S-6). Water droplets formed upstream were injected with yellow dye at the first K-channel, mixed and incubated through a serpentine region, and injected with black dye at the second K-channel. Adjusting the length and geometry of the channel between the two K-channel injections tunes reaction time.²² Moreover, tandem operations need not be identical, so droplets could first be injected with reagent at one K-channel, then split at the next K-channel, etc. As noted above, adjusting the pressure on one K-channel modifies the flow through every channel.⁴⁵ Therefore, careful attention will be necessary to design multistep devices to orient multiple K-channel geometries such that they operate under mutually compatible pressures; however, the versatility of this geometry allows for the potential for dynamic reconfiguration.

Contamination and Bidirectional Material Exchange

During injection and extraction, the droplet-K-channel fluid interface collapses temporarily. Droplets mix and exchange with the K-channel fluid in a mass transfer process governed by the pressure drop across the K-channel, diffusion, and convective turbulence during fluid merger and breakup. While any injection technique which transiently fuses droplets with a reagent stream will extract some fraction of droplet material into the injected fluid, the K-channel explicitly addresses the inter-droplet contamination that may result. Leveraging the continuous flow through the cross-channel, the K-channel significantly reduces the risk of contamination from re-injecting extracted material from one droplet into later droplets. In Figure 4A (Movie S-7), the fluid passing the K-channel decreases 85% in fluorescent intensity between injection events at 300 Hz, indicating efficient clearing of extracted material. When desired, this can be improved by decreasing droplet frequency or increasing cross-channel linear flow rate to replace a greater fraction of cross-channel volume. Other injection methods lack the direct capability to flush out accumulated contaminants between injection events.^{18,19,35} Though continuous replacement of fluid through the K-channel does increase reagent consumption, the versatility of the design allows for the reduction/elimination of cross-contamination depending upon the application.

Besides monitoring the net volume change per droplet, $V_{Net,Droplet}$, decoupling the volumes simultaneously injected, $V_{Injected}$, and extracted, $V_{Extracted}$, informs an understanding of droplet composition following K-channel operations:

$$\Delta V_{Net,Droplet} = V_{Injected} + V_{Extracted} \quad (6)$$

In order to quantitatively characterize simultaneous material exchange during K-channel operations, droplet volume changes and fluorescence intensity were measured for water injection into fluorescein droplets (Figure 4B). This information, coupled with Equation 6, was used to decouple injection and extraction components (Figure 4C):

$$[Droplet]_F = [Droplet]_0 * (V_0 + V_{Extracted}) / V_F \quad (7)$$

where $[Droplet]_F$ and $[Droplet]_0$ are the final and initial fluorescein concentrations in the droplet, and V_F and V_0 are the final and initial droplet volumes.

While the injection or extraction terms dominated at high or low pressures, respectively, performance at intermediate pressures included significant contributions from injected and extracted volumes. For instance, intermediate pressure conditions simultaneously added and removed approximately 20% of droplet material. Therefore, it is critical to account for bidirectional material exchange when designing droplet reaction sequences to ensure that intended reagent concentrations are reached. Notwithstanding, in cases where cross contamination or bidirectional material transfer are unacceptable risks, techniques like droplet fusion and droplet splitting are alternative strategies.

Biochemical Compatibility

Besides characterizing the physical responses of K-channel devices to internal and external perturbations, we also wanted to demonstrate the amenability of K-channel systems to biochemical droplet manipulations. Based on the extended dynamic range and ease of tuning for K-channel injections, we envision that K-channel devices could be especially suitable for precise measurement of enzyme kinetics.^{5,7} For example, K-channel devices can routinely scan across a range of substrate injection volumes into enzyme-containing droplets. Coupled to the rapid internal convection of in-droplet mixing, this approach should enable rapid determination of enzyme velocities.^{46,47} To this end, we confirmed the bio-compatibility of K-channel operations for this purpose by monitoring the action of β -galactosidase on K-channel-injected substrate (resorufin β -D-galactopyranoside) in a downstream incubation region (Movie S-8). As mentioned previously, the increased reagent consumption via K-channel flow may make this approach poorly suited to studying some enzyme systems, but careful assay design can minimize substrate consumption.

Superparamagnetic Bead Capture and Sample Washing

In characterizing performance and material exchange, it is clear that the K-channel offers significant versatility in modifying droplet size and composition. Up to this point, we have presented K-channel operations which interact non-selectively with droplet contents. However, some reaction sequences must include selective steps like concentrating samples, decanting supernatants, and buffer exchange. To these ends, magnetic beads present an opportunity for capturing samples on a functionalized solid phase subject both to fluid flow and to applied magnetic field. Recently examples have integrated on-chip magnetic fields to concentrate and manipulate bead-bound samples, typically by splitting droplets and collecting beads in one of the two daughter droplets.^{13,27–31,38,39} We have expanded magnetic bead capture to take advantage of the simple tunability (1:1 to 1:5 daughter droplet ratio) and high throughput (200–500 Hz) of K-channel droplet splitting.

The K-channel device was modified to incorporate an array of N52 magnets 250 μ m away from the main channel-cross-channel intersection (Figure 5A). As droplet-encapsulated superparamagnetic beads approached the K-channel, the magnetic force concentrated them toward one side of the droplet as:

$$F_m = (1/\mu_0) * (m * \nabla)B \quad (8)$$

where F_m is the magnetic force on a point-like magnetic dipole (bead), μ_0 is the magnetic permeability of a vacuum, m is the magnetic moment of the dipole (assumed to be saturated and position independent in the vicinity of the magnet), and B is the magnetic field.^{28,48}

When performing magnetic bead operations in droplets, uniform magnetic bead encapsulation is of particular interest. Bead encapsulation in the presence of a magnetic field has been shown to result in ordering and chain formation that lead to variance in per droplet bead abundance.²⁷ To this end, we displaced the droplet-forming flow focusing structure 1 cm from the leading edge of the magnet. A serpentine mixer directly upstream of encapsulation also contributed to the disruption of chain formation. Finally, we compensated for gravity-based bead settling and aggregation by shortening the tubing connection to the device (5 cm) and by adding Optiprep Density Gradient Medium (Sigma Aldrich) to the bead suspension. This decreased magnetic bead settling, and first passing beads through a 15 μm pore microfluidic device removed contaminants and large aggregates prior to use. Overall, 10 ± 3 superparamagnetic beads (1–3 μm diameter) were encapsulated per droplet.

As droplets approached the magnet, beads were selectively pulled into the lower hemisphere of the droplet (away from the K-channel intersection), and, after droplet splitting, the majority of beads were retained in the main channel. Daughter droplets flowed through a serpentine mixer to recirculate beads before passing into a detection channel region. Figures 5B–C provide representative examples of bead capture at the K-channel (Movie S-9) and at the detection channels (Movie S-10). Under these conditions, K-channel droplet splitting retained $96 \pm 1\%$ of magnetic beads when removing $50 \pm 1\%$ of droplet volume at a droplet frequency of 380 ± 20 Hz. (See Figure S-4 for a detailed description of magnetic bead capture analysis.) While some devices have reported larger droplet fractions removed (60–90%) or higher capture efficiencies (98–100%), K-channel magnetic separation proceeds at droplet frequencies more than an order of magnitude faster than these approaches (0.5–30 Hz).^{27–29} We anticipate that K-channel bead retention would be similarly improved by velocity reduction to increase the residence time for beads in the magnetic field. Ongoing work seeks to concentrate magnetic beads in smaller droplet fractions per splitting event.

Given the versatile range of K-channel operations, we expanded our K-channel devices to include a full sample washing mode. Figure 6 (Movie S-11) demonstrates how magnetic bead-laden droplets passing through the device (Figure 6A) split at the first K-channel (Figure 6B), are respaced at an oil channel (Figure 6C), and double upon water injection at the second K-channel (Figure 6D). By removing $46 \pm 2\%$ of droplet volume during splitting followed by injecting $93 \pm 7\%$ of remaining daughter droplet volume, the washing device effected a net concentration decrease of 48 % with only a net volume increase of 7 % across the device. Importantly, droplet washing at 200 ± 10 Hz successfully retained $98 \pm 5\%$ of encapsulated magnetic beads. While Electrowetting on Dielectric (EWOD) devices routinely manipulate small droplet populations or single droplets at a time through washing steps,⁴⁹ very few examples have interfaced this technique into continuously flowing droplets.^{38,39}

This K-channel approach provides a rapid (200 Hz), highly efficient (98% retention), and tunable washing strategy for continuously flowing droplet microfluidic systems.

CONCLUSION

Broadly speaking, methods for fluid manipulation within droplet microfluidics typically rely upon monofunctional device components that have limited capacity to be dynamically controlled. In contrast, the K-channel is a multifunctional tool that in a single design can accomplish a wide range of common droplet operations. Droplets can be injected into, extracted from, split, and respaced. Integration of a magnet even allows selective sample manipulation in droplet splitting and washing operations. As a modular building block, parallel and serial combinations of K-channels can be interfaced to perform sophisticated chemical and biochemical reaction sequences. Furthermore, we anticipate that K-channel's robustness, ease of tuning, and versatility in changing operations will be very attractive features, especially in using this device as a fundamentally enabling tool for rapid optimization of on-chip chemistry.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

K-channel device operation. Arrows indicate flow directions. After forming at a T-junction (left), droplets (blue) flow through the main channel to the K-channel element (right). The K-channel fluid (orange), an aqueous or an oil phase, flows through the cross-channel in an anti-parallel direction relative to main channel flow. An electric field may be supplied via the electrode channels (gray) to destabilize passing droplets. Through the interaction at the K-channel, droplet size, number, composition, and spacing can be altered.

Figure 2.

Multiple K-channel operations on a single device. Droplets flow left to right. The K-channel continuous phase flows right to left. (a) High K-channel pressure with electric field injects into droplets. (b) Low K-channel pressure with electric field extracts from droplets. (c) Moderate K-channel pressure without electric field maintains the droplet-K-channel interface. (d) Low K-channel pressure without electric field splits droplets under oil flow. (scale bars = 100 μm)

Figure 3.

K-channel operation characterization. (a) Net droplet volume change becomes more positive with increasing applied K-channel pressure. In the highlighted region, a stable droplet-K-channel fluid interface occurs in the absence of an applied electric field. (b) K-channel volumetric flow rate into droplets is directly proportional to applied pressure ($R^2 = 0.981$). (c) Increasing the K-channel inlet hydraulic resistance (by decreasing the inlet channel width from 40 μm to 25 μm to 15 μm) decreases net droplet volume change. (d) Increasing applied K-channel pressure reduces the droplet fraction removed during oil flow-induced droplet splitting.

Figure 4.

K-channel material exchange. During K-channel operations that merge droplets with the continuous aqueous phase, bi-directional exchange of material occurs. (a) The K-channel extracts fluorescein from droplets as it injects water. Continuous flow through the K-channel washes away extracted fluorescein to reduce the likelihood for droplet-to-droplet cross-contamination. The white arrow highlights the position of a single droplet across subsequent frames. (b) Monitoring the net change in droplet volume and fluorescein concentration at the K-channel enables (c) decoupling of the relative magnitudes of water injection, fluorescein extraction, and net volume change (the sum of injection and extraction) at each K-channel pressure. (scale bar = 100 μm)

Figure 5.

Magnetic bead capture. (a) Schematic of bead capture device. Arrows indicate flow directions. Magnetic bead (brown) containing droplets (blue) form at a flow focusing geometry (left) followed by droplet splitting under oil flow at a K-channel (middle). During droplet splitting, a magnet (dark gray) pulls beads into only one of the two daughter droplets. Electrode channels (light gray) are not enabled during this operation. After droplet splitting, waste droplets without magnetic beads (upper) and sample droplets with magnetic beads (lower) flow to the detection channels (right). (b) The nearby magnet pulls beads (circled in red) into one of the two daughter droplets during droplet splitting at the K-channel. (c) The detection channels show high incidence (96%) of magnetic beads in the sample droplets (lower channel) and low incidence (4%) of magnetic bead loss (white arrow) into waste droplets (upper channel). (scale bar = 100 μm)

Figure 6.

Magnetic Bead Washing. (a) Schematic of washing device. Light gray arrows indicate flow direction. After magnetic bead encapsulation in droplets (not shown) droplets (b) split at the leftmost K-channel under parallel oil flow, (c) respace at an oil channel, and (d) double in size upon injection at the rightmost K-channel using parallel water flow in an electric field (supplied by red electrode channels in schematic). Magnetic beads (highlighted by red arrows) are pulled toward the magnet (dark gray in schematic) and are retained during operations. (scale bars = 100 μm)