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Novel Microfluidic Architecture for 1:1 Pairings of Distinctly Sized Particles

A dissertation submitted in partial satisfaction of the  
requirements for the degree Master of Science

in

Engineering Sciences (Mechanical Engineering)

by

Spencer León Martín

Committee in charge:

Professor James Friend, Chair  
Professor Ben Croker, Co-Chair  
Professor Abhishek Saha

2026



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University of California San Diego

2026

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Chapter 2, in part is currently being prepared for submission for publication of the material. Friend, James; Salari-Namin, Sahand; and Croker, Ben, 2026. The dissertation author was the primary researcher and author of this material.



## ABSTRACT OF THE DISSERTATION

Novel Microfluidic Architecture for 1:1 Pairings of Distinctly Sized Particles

by

Spencer León Martín

Master of Science in Engineering Sciences (Mechanical Engineering)

University of California San Diego, 2026

Professor James Friend, Chair  
Professor Ben Croker, Co-Chair

Understanding and dissecting the dynamics of single-cell interactions is integral to immunological research. However, many of the microfluidic platforms that seek to facilitate this are constrained by strict particle size limits, low trap density, or complex multilayer fabrication processes. This thesis presents a microfluidic flow-reversal platform with a high-density trap array that hydrodynamically pairs two distinctly sized particle populations in a 1:1 ratio. Fabricated as a single layer via conventional soft lithography, the device incorporates 1,566 bidirectional traps that enable sequential loading of particles. Initially motivated by investigating host-pathogen interactions between neutrophils and *Coccidioides* spherules, the device was characterized using

10  $\mu\text{m}$  and 20  $\mu\text{m}$  fluorescent beads as model particles across operational flow rates from 75 to 200  $\mu\text{L}/\text{min}$ . The results demonstrate an optimal 1:1 capture yield exceeding 40% at 125  $\mu\text{L}/\text{min}$ , corresponding to over 626 paired sites per chip. This platform serves as a high-throughput and easily fabricated framework for single-cell interaction assays with broad research applicability.

# Chapter 1

## Background

### 1.1 Introduction

The field of immunology finds its basis in categorization and analysis of a host's reaction to pathogens, whether this be the inspection of macro effects on a host's immune system or the changes at the cellular level. The latter domain has expanded significantly alongside bespoke technologies increasing the resolution and capabilities of existing analytical tools [1, 25].

Immunology historically relied on bulk assays to determine the effect of a disease or medicine, co-culturing populations and measuring ensemble averages as results. While these methods provided critical insights, the averages systematically obscured edge cases and evidence of cellular heterogeneity. With the development of the first labs-on-a-chip, these constraints were mitigated by the ability to precisely manipulate fluids and cell populations on the micron scale, capable of isolating individual cells [2, 57, 41].

One notable pathogen is *Coccidioides*, the cause of the infectious respiratory fungal disease called *Coccidioidomycosis*, commonly referred to as “Valley Fever”. It is endemic to dry warm regions such as the southwest of North America and several separate regions in South America [5, 19]. Although infections can often be mild, the clinical course can worsen and potentially culminate in life-threatening conditions for immunocompromised hosts. However, climate change has been recently found to enable further spread of the disease [26]. Valley Fever has no definitive cure and therapeutic options remain limited, often requiring extended periods

of anti-fungal treatment if conditions worsen [17, 52, 26]. These circumstances have motivated further research into *Coccidioides* spherules and their interactions with immune cells in the hopes to develop more therapeutic options and better understand the disease.

Microfluidic platforms have emerged as a promising platform for accomplishing such investigations, offering precise control and capture of cells with minimal fluid volumes [57]. Over the past 20 years, microfluidics has found application in cell sorting, sequencing, drug screening, chemotaxis analysis and cell-cell interaction facilitation [41].

Of architectures designed to mediate cell-cell interactions, hydrodynamic trapping methods are uniquely suited to the task, offering deterministic spatial positioning and robust immobilization of cells. This is done through pressure-driven flow and constraining geometry designed to capture single cells via localized fluidic resistance with passive flow through the device. Use of such designs are particularly advantageous due to the lack of reliance on external electrical, magnetic, or optical forces [35].

The present research focuses on a novel hydrodynamic trap array design that was engineered for these objectives. The architecture leverages flow reversal to capture two size-varying cell populations; the intended use being to monitor interactions between primary immune effectors (neutrophils) and *Coccidioides* spherules.

## **1.2 Bulk Assay Methodologies**

A host's immune response is defined by coordinated actions between immune cells and invading pathogens. Neutrophils are the most abundant of immune cell responders, reacting with phagocytosis or degranulation for pathogen clearance or containment [27, 46, 36]. These reactions involve direct physical engagement between the neutrophil and pathogen, ranging from brief contact to sustained phagocytic capture. As such, the study of these reactions is paramount to better understand the intricacies of the immune system in disease progression and immune regulation.

Historically, the studies of such interactions have been done using bulk population analysis using techniques such as enzyme-linked immunosorbent assays (ELISA) [29, 15], polymerase chain reaction (PCR) [42], and western blotting [53]. Migration assays, such as those performed with Boyden chambers and transwell systems, utilize chemical gradients to drive cell migration through a porous material using chemotaxis induced by chemical gradients [62, 61, 4]. This method succeeds in manipulating immune cells in a suspended environment, but lacks the precise controls required to isolate individual cells, much like previous examples.

Though these methods are effective for cell populations, such as measuring large-scale protein release, these methods output an average of the cells' expressions. This averaging obscures the inherent heterogeneity of cells and potentially unique responses to stimuli [2, 21, 37]. Even genetically identical cells can exhibit different signaling responses or pathogen clearance capabilities [45, 6, 18].

These differences are especially important with host-pathogen interactions. Apart from immune cells' abilities varying substantially from cell to cell, pathogens have also been seen to have a wide range of morphology or stress response among a population [45]. Consequently, bulk assays lack the spatial resolution required to analyze the stochastic nature of cell-cell interactions.

*Coccidioides* spherules in particular are an important case to study because of their large, endospore-containing spherules that actively evade or resist normal immunological reactions [17]. Despite neutrophils being the primary leukocyte recruited during coccidioidomycosis, the discrete kinetics between these effectors and the pathogen interacting are not yet fully understood [23]. Thus, further, more precise study of such interactions may yield important insight into *Coccidioides*' resilient nature to existing treatment and immune responses.

### **1.3 Precision Control Methodologies**

Before the development of microfluidics, macroscale assays predominated immunological research and cell sorting. High-throughput flow cytometry is a foundational example of precision

cell analysis that characterizes single-cells by passing them sequentially through a laser beam to detect and analyze light scattering and fluorescence emission. When paired with fluorescent markers and fluorescence-activated cell sorting (FACS), this platform achieves rapid phenotypic profiling and physical isolation of each target cell [22]. However, because flow cytometry requires cells to be isolated during analysis, it can't measure specific cell-cell caused reactions or separate pairs.

Microscopy-based methods also offer a high-resolution alternative to bulk assays, using time-lapsed fluorescence imaging to track cell chemotaxis and phagocytosis over extended durations [49]. While this approach balances population-level data with single-cell resolution, it fundamentally lacks any physical means of cellular manipulation. Standard imaging wells are moreover unable to isolate, localize, or otherwise fluidically control cells. Consequently, deterministic mediation of cell-cell interactions or precise alteration of the local chemical environments cells are exposed to in real time remains heavily constrained.

Prior to the widespread adoption of microfluidics, several high-precision manipulation techniques were developed for the robust manipulation and study of single cells. Optical tweezers [3] exemplify these approaches, utilizing focused laser beams to trap and move individual cells or pathogens with high spatial resolution. Similarly, dual-pipette aspiration methods physically position cells in close proximity using finely controlled micropipettes to measure adhesion forces or characterize responses during cellular contact. While these methods of physical manipulation do offer extreme precision over cell pairs, accumulating extensive datasets remains highly labor-intensive, resulting in low throughput. Thus, collecting statistically robust datasets demands prohibitive operation times.

Despite providing critical insights, conventional optical and manual high-precision methods exhibited an inverse relationship between the level of experimental control and the throughput required to evaluate extensive sample sizes. Addressing these limitations required platforms capable of evaluating single-cell behavior within highly controlled environments, where chemical gradients and spatial positioning could be independently manipulated.

## 1.4 Emergence of Microfluidics

Microfluidics refers to the manipulation of fluid volumes within channels with dimensions on the order of micrometers. The discipline emerged from microfabrication innovations in the semiconductor industry in the late twentieth century [57]; these techniques, adapted from photolithographic fabrication processes, enabled the development of early microfluidic chips. These foundational platforms demonstrated the capacity to perform chemical and biological assays with minimal fluid volumes and high precision.

A defining characteristic of these microchannels is the dominance of the laminar flow regime governed by the low Reynolds numbers inherent to them [48]. Without turbulent mixing, flow in microfluidic devices is highly predictable and deterministic, facilitating the establishment of chemical gradients or the finely tuned transport of particles in the flow. These flow characteristics benefit biological experiments specifically requiring highly controlled environmental conditions. In addition to precise spatial manipulation achievable with fluid, the microscale channels of these devices also accelerate heat and mass transfer across the whole chip [12]. The scale and footprint of the devices permits the integration of multiple experimental functions on a single substrate. Despite these benefits, microfluidic platforms face inherent structural and operational limitations, including specialized fabrication workflows, susceptibility to device clogging, surface adsorption of biomolecules, and sampling or throughput constraints. Subsequent development has focused on mitigating these constraints to expand analytical throughput and enhance the platform's versatility.

Polydimethylsiloxane (PDMS) is established as one of the most widely used materials in biological microfluidics because of its optical transparency, gas permeability, low cost, and compatibility with soft lithography fabrication methods [33]. Device fabrication utilizes a master mold generated via UV exposure, development, and curing of photoresist on a silicon wafer, against which the PDMS is cast, thermally cured, and separated from the mold to isolate individual chips. This enabled rapid prototyping of complex microchannel geometries and

significantly accelerated growth within the field.

Microfluidics has rapidly expanded into numerous biomedical applications such as organ-on-chip systems, droplet microfluidics, single-cell sequencing, and cell sorting technologies [41]. A critical subset of these platforms focuses on the high-throughput, simultaneous immobilization of multiple individual cells through constraining geometry and passive fluid flow.

## **1.5 Single-Cell Analysis and Microfluidic Cell Capture**

Single-cell analysis is a prominent subject of research due to inherent cellular heterogeneity in gene expression, metabolism, migration, and immune function in a phenotypically uniform population [50]. Microfluidic systems are the ideal platform for such investigations due to their microchannel dimensions aligning the characteristic dimensions of biological cells.

Methods for single-cell capture and manipulation can be categorized into two main modalities: active and passive approaches. Active methods rely on external forces introduced into the microfluidic system, such as optical tweezers, magnetic forces, dielectrophoresis, or acoustic waves [3, 56, 59]. Although capable of highly selective manipulation, these platforms often require complex instrumentation and integration while potentially inducing additional stress on the cells under study.

However, passive methods exploit intrinsic channel geometry and fluidic flow paths. Prominent modalities include deterministic lateral displacement, inertial focusing, hydrophoresis, and hydrodynamic trapping [10]. Passive trapping designs typically also offer simplified fabrication and operation workflows. While hydrodynamic shear forces and restrictive channel geometries are still capable of deforming or losing relatively sensitive cells like neutrophils, optimized passive designs can be low-stress methods for maintaining cell viability and long-term immobilization.

Among these, hydrodynamic trapping captures cells or particles as they flow through the constrictive geometry in the device. As a result of its efficacy, hydrodynamic trapping systems



have been applied to a variety of investigations, including the isolation of stem cells, bacteria, and tumor cells, as well as deterministic cell-cell pairing of heterogeneous cell populations [47, 16, 34]. The ability to both maintain continuous perfusion and immobilize strategically spaced arrangements of cells makes this method a highly viable option for extended experiments and time-lapse imaging [9].

## **1.6 Hydrodynamic Trapping Mechanisms**

Hydrodynamic trapping hinges on the microscale fluid dynamics of pressure-driven flow. In such low Reynolds number conditions, viscous forces dominate over inertial forces, enabling precise and predictable flow streamline simulations [48]. The suspended cells' adherence to these predictable trajectories at this flow regime can be exploited to guide cells into purpose-designed traps.

Mechanistically, hydrodynamic traps operate by leveraging differences in flow resistance between the restrictive geometry around the capture sites and adjacent bypass channels. Initially, fluid selectively routes through an unoccupied trap via a gap designed to allow such flow. However, when a cell occupies the trap, the previously open gap becomes occluded, significantly increasing the flow resistance relative to the bypass channel. As a result, subsequent cells are diverted to the bypass channel and populate further traps downstream [20]. This loading mechanism scales effectively across micro-arrays for deterministic single-cell capture [9].

Though the principles of trapping cells remain constant, a variety of trap and array geometries have been developed to optimize capture efficiency for different experimental parameters and procedures. Conventional configurations include cup-shaped traps, U-shaped traps, constriction based systems, and weir structures [35, 16, 34]. Each design aims to minimize fluidic shear stress while producing localized conditions to encourage cell capture.

Different trap designs have also been made to accommodate paired-cell capture to study kinetic processes such as immune interactions, fertilization, and intercellular signaling

[47, 13, 16]. Sequential loading is a predominant method for populating traps deterministically; this involves capturing the primary cell type first before capturing the second type with some modified flow environment. Flow reversal represents a classic strategy to accomplish paired-cell trapping. This approach requires alternating the direction of flow to move cells from an initial capture site to the forward end of another that is capable of holding both cells. This method is highly effective for investigations with immune interactions where immune cells and pathogens have a wide range between sizes. However, many current applications of these systems are limited in throughput, trap density, and compatibility with differently sized cells [28, 60, 44, 40].

## **1.7 Single-cell and Pair-Wise Hydrodynamic Trapping**

Recent pair-wise trapping studies have focused on capture efficiency, imaging throughput, and the deterministic control of cell interactions. An early demonstration of cell-cell trapping was reported by Skelley et al., using microfluidic docking structures to pair mammalian cells for a cellular fusion study [47]. Subsequently, Dura et al. expanded on this field by trapping pairs of lymphocytes in predesignated configurations using flow reversal techniques to study long-term signaling [13].

Additional efforts towards scaling the size of the trapping arrays were made, but maintaining uniform trap occupancy remained challenging. Lecault et al. achieved a high-throughput microfluidic culture array for hematopoietic stem cells; however, occupancy across these large arrays remained variable and subject to fluidic resistance balancing and uniformity [28]. Similar limitations are observed in dynamic trapping arrays developed by Di Carlo et al., with traps further upstream in an array being preferentially populated due to pressure redistribution effects [11]. Such inconsistencies reduce effective throughput and weaken scalability for larger statistical studies.

Pairing cell populations with distinct geometric and structural disparities introduces significant additional operational challenges. While many pair-wise trapping platforms were

optimized for homogenous cell populations, handling dissimilarly sized cells fundamentally compromises trap efficiency [60]. Sequential loading methods mitigate this control issue by introducing each cell population independently, but many of these systems rely on multilayer valve integration or smaller array sizes [55, 44]. Large-scale single-cell trapping and imaging using microwell arrays has also demonstrated the potential for high-throughput individual cell isolation, but these approaches differ from hydrodynamic pairing architectures in their mechanism of cell capture [39].

The trap design presented in this work addresses several of these limitations by achieving increased trap density and quantity; the flow-reversal design having geometry created to accommodate differently sized cells such as *Coccidioides* spherules and neutrophils.

## **1.8 Current Limitations and Research Motivation**

Despite advancements in hydrodynamic trapping technology, some limitations persist. For example, many designs prioritize capture efficiency or total analytical throughput but struggle to optimize for both simultaneously. In addition, a relatively small number of systems are specifically designed for the pairing of highly asymmetric particles within high-density arrays.

Furthermore, maintaining deterministic single-cell trapping efficiency requires mitigating channel clogs and elevated fluidic shear stress. Immune cells, such as neutrophils, are particularly susceptible to mechanical stresses induced by fluid flow or restrictive geometry, necessitating structural optimization of flow speeds and trap shape [46, 36].

While established paired-cell trapping systems do exist, they frequently necessitate complex loading procedures or external actuation methods. As such, a passive platform capable of robust and reproducible results would greatly improve the accessibility of intercellular biological studies.

The presented research seeks to address these operational challenges through the development of a high-density, flow-reversal hydrodynamic trapping array optimized for the isolation of

highly asymmetric cellular pairings. Specifically, this platform facilitates the deterministic capture of neutrophils and *Coccidioides* spherules to scale the throughput of controlled, one-to-one host-pathogen interactions. By maintaining the cells immobilized long-term under continuous perfusion, this system enables the sustained observation of cellular kinetics at the single-cell level.

## 1.9 Conclusion

Immune-pathogen interaction studies are increasingly focusing on mechanisms that require systems capable of resolving microscale cell behavior. While immunological bulk assays provide valuable macroscopic data, they intrinsically obscure cellular heterogeneity and the spatial nuances of physical interactions between individual cells [37].

Microfluidics platforms have served to answer most of these challenges, providing precise control over microchannel environments, perfusion, and spatial cell positioning. Among the numerous microfluidic trapping methods, passive hydrodynamic designs are distinctly advantageous for single-cell studies owing to their simplicity, scalability, and low impact on relevant biological samples.

Hydrodynamic trapping systems have proven effective over prolonged observation windows for both single and paired-cell trapping across a number of applications [13]. Despite this, existing methods struggle with simultaneous optimization of trap density and the capacity for size-variable cell capture.

The presented research seeks to improve upon these operational constraints by iterating on contemporary microfluidic trapping devices. This objective is achieved through the implementation of a high-density, flow-reversal hydrodynamic trapping array optimized for the capture of differently-sized cell populations, establishing a high-throughput framework for the long-term characterization of single-cell host-pathogen kinetics.

# Chapter 2

## Research

### 2.1 Introduction

Understanding how immune cells recognize, engage, and eliminate pathogenic targets is key to explaining host-pathogen interactions and treatment development [7, 1, 24]. Single-cell level observation of such encounters has provided critical insight into host defence from cancerous, viral, bacterial, and fungal infections. Investigation into these inherently heterogeneous cell interactions and behavior requires profiling tools capable of pairing white blood cells and a broad spectrum of pathogens and target cells of diverse sizes and properties [7, 1, 24].

One key clinical application for this resolution of observation is the interactions between host neutrophils and the fungal pathogen *Coccidioides*. *Coccidioides* is the cause of coccidioidomycosis (Valley Fever) and is endemic to arid regions of the southwest of North America and several separate regions in South America. Recent studies suggest that environmental changes spurred by climate warming have contributed to an expansion of the pathogen's geographic range, increasing disease incidence [38]. Consequently, an in-depth analysis of neutrophil-*Coccidioides* interactions to determine pathogen engagement and immune evasion is of growing importance.

However, because of the multi-hour window of the interactions' time and the necessity of the cell pairings' isolation, conventional methods such as flow cytometry would be limiting. Conventional bulk assays, while high-throughput, average the behavior of thousands to millions of cells and, therefore, can obscure rare or edge-case reactions that could be clinically significant

[54, 8, 43]. In addition, the temporal evolution of pairings is critical for characterization of heterogeneous behavior and standard bulk methodologies only capture population-level readouts. As a result, population assays are fundamentally insufficient for capturing the highly-variable dynamics of interest between the two cell populations. Alternatively, interactions can be induced through the approximation of individual cells using optical tweezers or dual pipette assays with high reliability and precision. Conversely, both methods rely on forming a single cell-cell pairing at a time, thus making them statistically underpowered and intractable for generating the high-volume data sets required for capturing rare phenotype reactions.

Microfluidic platforms have emerged as powerful tools for studying single-cell behavior due to their ability to isolate and manipulate individual cells in a controlled environment [30]. Within this field, paired-cell trapping devices enable direct observation of cell-cell interactions such as pathogen engagement and therapeutic screening [13]. Existing cell-pairing strategies such as hydrodynamic traps [44], droplet encapsulation [31], and microwells [51] have specific use cases, but remain constrained by several limitations. While these architectures improve on the resolution constraints of bulk assays, they remain limited by their effective throughput and trap density, again restricting the statistical power needed for mapping the sought rare heterogeneous reactions. Furthermore, many existing microfluidic systems suffer from strict size and property selectivity [47, 14], requiring cells of similar dimensions and deformabilities. Of the few designs without such restrictions, fabrication or operational complexity, such as multilayer valve integration, is often present. When pairing rigid 20  $\mu\text{m}$  diameter *Coccidioides* spherules with highly deformable 8  $\mu\text{m}$  diameter neutrophils, existing flow-reversal and pairing methods fall short for this application.

Thus, we propose a high-density, hydrodynamic microfluidic trapping architecture designed to maximize throughput while maintaining a simpler single-layer mold fabrication. This architecture, hereafter referred to as the flow-reversal device, enables the capture and pairing of two differently sized particle populations through sequential loading with flow-reversal and bidirectional traps. Its design only requires a single layer and no active components beyond flow

control while still handling highly asymmetrical particle combinations. To validate this method, the flow-reversal device was rigorously characterized using microbeads; trapping over time and flow-rate-dependent performance were measured across a range of conditions.

## **2.2 Device Design**

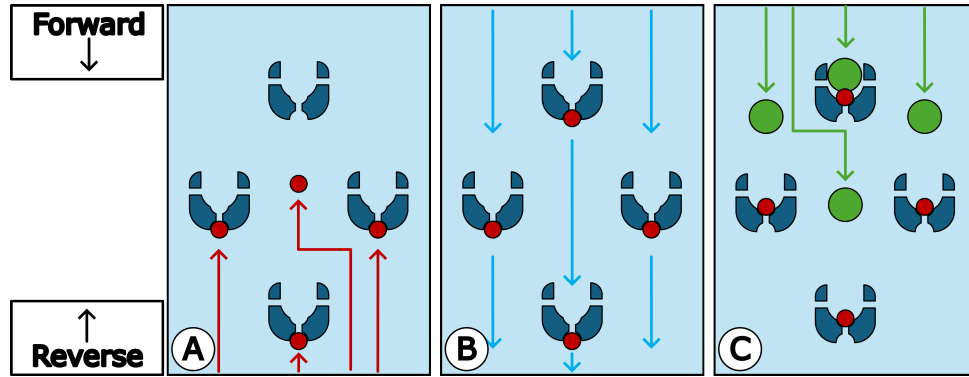
### **2.2.1 Geometry**

The flow-reversal device was developed to facilitate the controlled pairing and long-term observation of highly asymmetric cellular species, particularly focusing on host-pathogen interactions. The original motivation came from investigating interactions between *Coccidioides* spherules and neutrophils, whose diameter difference presents incompatibility with existing cell-pairing methods. Though existing cell-trapping methods do permit adequate spatial control and long-term immobilization for imaging, their throughput and requirement for cells to be of similar size or mechanical properties preclude their utility for this application. Consequently, we developed a trap architecture capable of particle capture purely through hydrodynamic forces and a single flow-reversal step.

The microfluidic device is fabricated from a polydimethylsiloxane (PDMS) substrate bound to a glass coverslip. The architecture features a high-density trapping array with free-standing traps arranged in alternating staggered rows. Each individual trap has two distinct capture zones located at the anterior and posterior poles, connected by a small gap that allows flow through it when unoccupied to prevent clogging and promote particle capture. The channel height is uniformly 22  $\mu\text{m}$  throughout the device, accommodating particles with diameters ranging from 10 to 20  $\mu\text{m}$ . During experiments, flow is controlled through the positive pressure of the injection mode of a standard syringe pump.

After preliminary live testing with the previous design, seen in Figure 2.2, and finding feasibility, the array spacing was iterated upon to address clogging and density concerns. The individual trap footprint has been reduced while inter-trap spacing has been increased, achieving

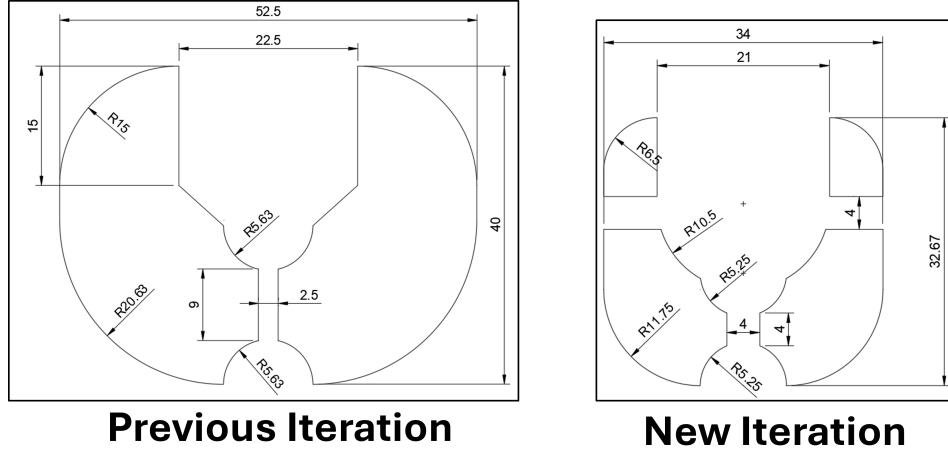
a higher overall array density despite the wider pitch. The spacing between traps of  $32\ \mu\text{m}$  horizontally and  $40\ \mu\text{m}$  vertically ensures unobstructed traversal for the larger  $20\ \mu\text{m}$  diameter particles. These traps also have additional gaps in the side of their front trapping region, allowing for more flow through the trap before the larger particle is captured. Integrated support pillars are distributed throughout the channels to maintain an aspect ratio below 10:1 (span-to-height), preventing roof collapse or structural sagging [58].



**Figure 2.1.** A) Red  $10\ \mu\text{m}$  beads getting caught in the reverse end of the trap. B)  $10\ \mu\text{m}$  beads move to the trap directly beneath them when the flow direction switches. C) Green  $20\ \mu\text{m}$  beads are input into the chip and trapped in the forward end of the traps.

The key active component of the sequential trapping is the flow reversal, depicted in Figure 2.1. Initially, the reverse end of each trap is filled with one smaller particle through reverse flow. After the array is sufficiently populated, the flow direction is reversed, causing the trapped particles to be displaced from their initial capture sites into the larger, unpopulated regions of their respective downstream traps. Once repositioned, the second larger particle population is flowed through in the forward direction, getting captured in the front end of each trap already populated by one smaller particle, forming deterministic 1:1 pairs. After both particles occupy the trap, the hydrodynamic resistance of the area around them increases, rerouting additional incoming particles further downstream while also keeping the trapped particles in their trap with pressure from the flow.





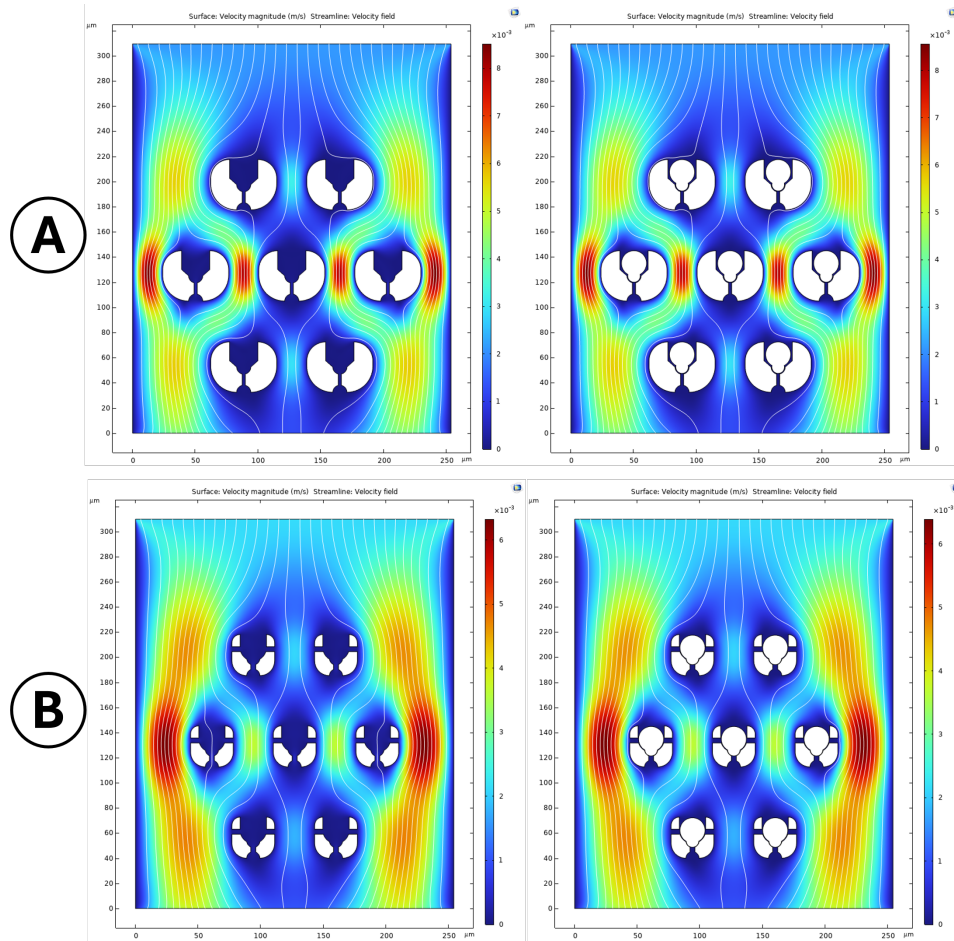
**Figure 2.2.** Previous design for pair-cell trap (left) and new, presented design (right). Notable changes include side gaps, reduced parallel footprint, widening of gaps, and less unused space. Measurements done in micrometers.

## 2.2.2 Simulations

Isopropanol (IPA) was selected as the working fluid due to its low surface tension, which facilitated spontaneous bubble clearance in the device during operation, as well as its surfactant-like effect in preventing aggregation of polystyrene beads. Consequently, surfactants such as bovine serum albumin (BSA) or Tween-20 were omitted from the fluidic medium.

The finite element method (FEM) was implemented in COMSOL Multiphysics v.6.4 to qualitatively validate trap functionality and inter-feature spacing optimizations (Figure 2.3). The modeled domain comprised a representative sub-array containing seven traps within a scaled fluidic chamber, utilizing isopropanol (IPA) as the working fluid to evaluate both boundary-wall and inter-feature hydrodynamics. This restricted computational domain enabled the tractable application of an extremely fine global mesh to ensure numerical accuracy. Fluid velocity profiles were derived by solving the steady-state, incompressible Navier-Stokes and continuity equations for a Newtonian fluid:

$$\rho(\mathbf{u} \cdot \nabla)\mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + \mathbf{F}$$



**Figure 2.3.** COMSOL Multiphysics simulation of fluid velocity through unoccupied and occupied traps for the previous design (A) and current design (B). Streamlines show sharp turns prior to unoccupied traps, frequently causing cells trapping due to their inability to follow the severe movement.

$$\nabla \cdot \mathbf{u} = 0$$

where  $\mathbf{u}$  represents the fluid velocity vector ( $\text{m} \cdot \text{s}^{-1}$ ),  $p$  is the static pressure (Pa),  $\rho$  is the fluid density ( $\text{kg} \cdot \text{m}^{-3}$ ),  $\mu$  is the dynamic viscosity ( $\text{Pa} \cdot \text{s}$ ), and  $\mathbf{F}$  represents the body-force density ( $\text{N} \cdot \text{m}^{-3}$ ). External body forces (gravitational, magnetic, and buoyant) were assumed to be negligible ( $\mathbf{F} = \mathbf{0}$ ). The working fluid was modeled as pure IPA with a density of  $\rho = 768 \text{ kg} \cdot \text{m}^{-3}$  and a dynamic viscosity of  $\mu = 2.04 \text{ Pa} \cdot \text{s}$ . The inlet boundary condition was specified as a mass flow rate, calculated from a volumetric flow rate of  $100 \mu\text{L} \cdot \text{min}^{-1}$  scaled by the fluid density, while the outlet was assigned a zero-static-pressure boundary condition ( $p = 0 \text{ Pa}$ ). Solid device boundaries were assigned a no-slip condition ( $\mathbf{u} = \mathbf{0}$  at the wall), and captured microparticles were modeled as fixed, rigid, non-deformable geometric boundaries.

The final device contains 1,566 trap sites arranged in a compact footprint, enabling a large number of simultaneous particle-particle interaction observations. This high throughput architecture is particularly suited for observing and characterizing rare or heterogeneous reactions between particle populations on the single-particle level over long time spans.

## 2.3 Fabrication

The microfluidic device was fabricated via standard soft lithographic techniques at the Nano3 cleanroom facility (University of California, San Diego). The master mold was patterned on 100 mm (4-inch) silicon substrates. Wafers were cleaned, spin coated with  $22 \mu\text{m}$  of SU-8 3025, and patterned with a maskless aligner (MLA 150, Heidelberg Instruments); relevant processing parameters are summarized in Table 2.1. Notably, direct-write laser exposure required energy doses approximately double those typically applied in contact mask aligners. Due to the maskless nature of this system, CAD layout files were directly imported for photolithography without physical photomasks.

Following mold fabrication, the mold is then treated with an anti-sticking vapor to facilitate mold detachment when casting. Polydimethylsiloxane (PDMS, Sylgard 184) was then

mixed with a 10:1 weight ratio between base and curing agent and cast over the master mold. After degassing the mixture with a vacuum to remove trapped air bubbles from the casting, it was thermally cured overnight. Once cured, the PDMS was removed from the mold, diced into individual devices, and bound to glass using a UV/ozone cleaning system before a final bake.

The resulting chips are optically transparent through both the PDMS and glass slide, accommodating inverted or upright microscopy. Furthermore, the single-layer architecture lowers delamination risks and operational defects during fabrication. A comprehensive fabrication protocol is provided in Appendix A1.1.

**Table 2.1.** A list of fabrication parameters for the mold creation. Most values are derived from existing documentation for the photoresist.

| Parameter               | Device Specification                                   |
|-------------------------|--------------------------------------------------------|
| Thickness               | 22 $\mu\text{m}$                                       |
| Spin Speed              | 500 rpm at 100 rpm/s to 4000 rpm at 300 rpm/s          |
| Pre-Exposure Bake Time  | 3 minutes at 65°C, then 15 minutes at 95°C             |
| Exposure Dose           | 650 mJ/cm <sup>2</sup>                                 |
| Post-Exposure Bake Time | 1 minute at 65°C, then 5 minutes at 95°C               |
| Development Time        | 8 minutes                                              |
| Hard Bake               | 1 minute at 65°C, 1 minute at 95°C, 5 minutes at 150°C |
| Vapor Treatment         | 10 minute FDTs exposure, 10 minute water exposure      |

## 2.4 Experimental Methods

### 2.4.1 Experimental Setup

All chips used during experiments were PDMS cast from the master mold of each design onto a 25x75 mm glass substrate. The microfluidic chips used were connected to 0.5 mm ID, 1.5 mm OD Tygon tubing via 23 gauge steel pins and the flow was kept stable using a dual-syringe 11 Pico Plus Elite syringe pump at a constant flow rate. Tubing between the pump, waste reservoir, inlet and outlet of the chip was connected to a diagonal 4-way valve, used to control flow direction. The waste reservoir connected to the chip's outlet was a container capable of containing the total output fluid of the experiment. The experiments were conducted using

an Olympus BX51 microscope with 4x and 10x objectives, while a Swiftcam 10 Megapixel camera was used for image and video acquisition. Fluorescent excitation was done using the microscope's built-in systems. Experimental data presented in Section 2.5 were generated using 4 independent batches of devices, all fabricated from a single master mold. Each fabrication batch yielded 12 devices, resulting in 48 total chips; 36 devices were used for experimental evaluation while the remaining 12 chips were unused. Each experiment was conducted on a pristine chip, yielding 30 total chips that successfully contributed to the data set, 5 chips for each of the 6 flow rates, and 6 total chips that failed during operation and were excluded from the data.

### **2.4.2 Protocol**

Before experiments, the tubing and microfluidic chip were flushed with IPA to remove residual air bubbles from inside the system. Following this, the smaller bead type is flowed into the device at 75-200  $\mu\text{L}/\text{min}$  for five minutes. Two additional minutes of particle-free flow at the previous rate follows this to remove excess beads. After achieving sufficient population of the reverse sites, the 4-way diagonal valve is turned to reverse the flow direction, moving captured particles to the front capture site of their corresponding traps. The larger particle type was then flowed in at the corresponding flow rate until sufficient population. Another two minute cleaning period follows loading. After bead pairing is complete, a maintenance flow rate of 25  $\mu\text{L}/\text{min}$  was used to continue immobilization during additional imaging. A more comprehensive protocol and detailed description of the experimental procedure can be found in Appendix A2.

### **2.4.3 Data Analysis**

Images and videos were acquired throughout the loading process to evaluate the efficiency of each experimental step. During the reverse loading phase, video microscopy was acquired to quantify both the capture efficiency and the spatial progression of trap occupancy throughout the array. After loading was complete, a still image was acquired to quantify initial loading occupancy and provide the baseline image for calculating swap efficiency. Following bead

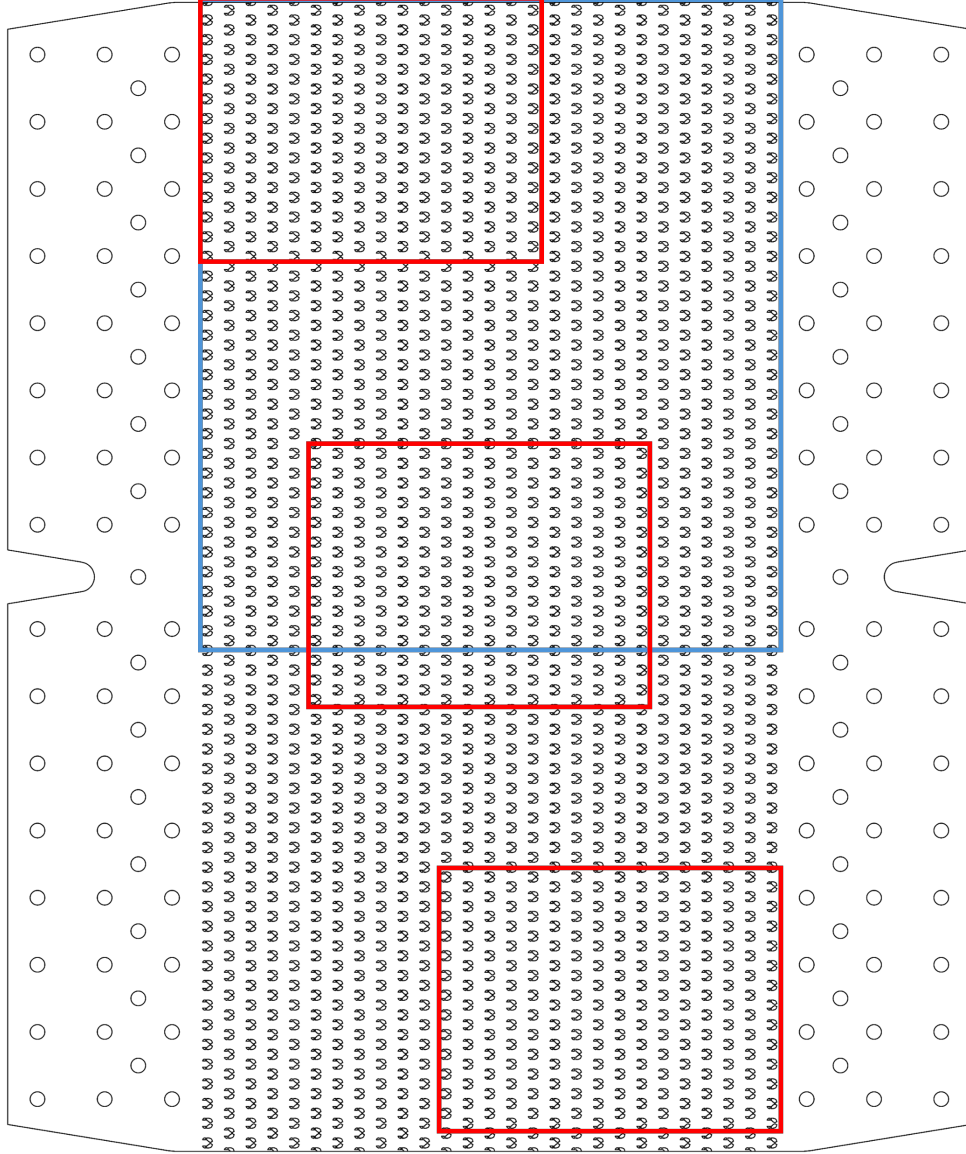
repositioning, a subsequent picture was captured to yield paired before-and-after images for quantifying swap efficiency. This same video recording and subsequent imaging protocol was repeated during forward loading to obtain matching metrics across both loading sequences.

Capture efficiency was calculated as the fraction of occupied traps relative to total traps in predesignated regions of the trap array (Figure 2.4) at predesignated timepoints during respective particle loading phases. Similarly, swap efficiency was calculated as the fraction of previously populated traps that successfully were recaptured by the corresponding downstream trap. After dual-particle loading was complete, the pairing performance in the three predesignated regions were imaged, assessed, and averaged to determine the representative pairing performance of the whole array. The collected videos were subsequently analyzed with custom python scripts using both OpenCV and Matplotlib to determine reverse and forward capture efficiencies, swap efficiency, spatial dependence on loading over time, and bead pairing distributions throughout the device. Additional details regarding the analytical methods used to differentiate microparticle populations are provided in Appendix A3.

## **2.5 Results and Discussion**

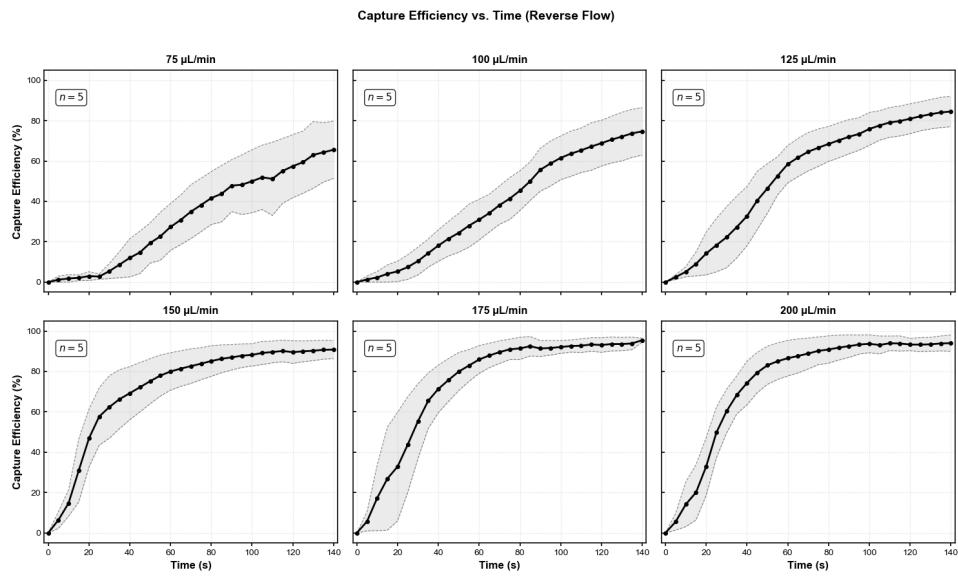
### **2.5.1 Bead Loading**

In order to characterize the performance of the flow-reversal device, microbeads were used to test its performance across a range of flow rates. Reverse loading, forward loading, particle repositioning, spatial occupancy development, and bead pairing efficiencies were each independently evaluated to quantify device capabilities. Loading in both orientations was monitored via video microscopy and analyzed to measure capture efficiency as a function of time. Video analysis of loading provided both capture rate over time and the means to determine spatial loading progression differences between flow rates. Dual-bead occupancy was evaluated to quantify the frequency of successful heterogeneous particle pair formation, serving as a proxy for the particle-particle pairing application that motivated the design.



**Figure 2.4.** Top-down view of trap array. Left here is considered the forward direction while right is the reverse direction. The red boxes show the fields of view used for bead pairing results, each containing 208 traps. The blue box is the region recorded for loading and swap efficiencies, containing 877 traps.

Bead loading in the reverse direction was first measured, with mean performance shown in Figure 2.5. Higher infusion rates exhibited rapid initial capture followed by an asymptotic plateau, whereas lower flow rates demonstrated a more linear progression in occupancy with reduced overall efficiency. Flow rates between 150 and 200  $\mu\text{L}/\text{min}$  achieved an average final capture efficiency exceeding 90% after 130 seconds of flow. The asymptotic trend of the capture efficiencies is consistent with behavior expected with progressively fewer available trap sites remaining as occupancy rises.

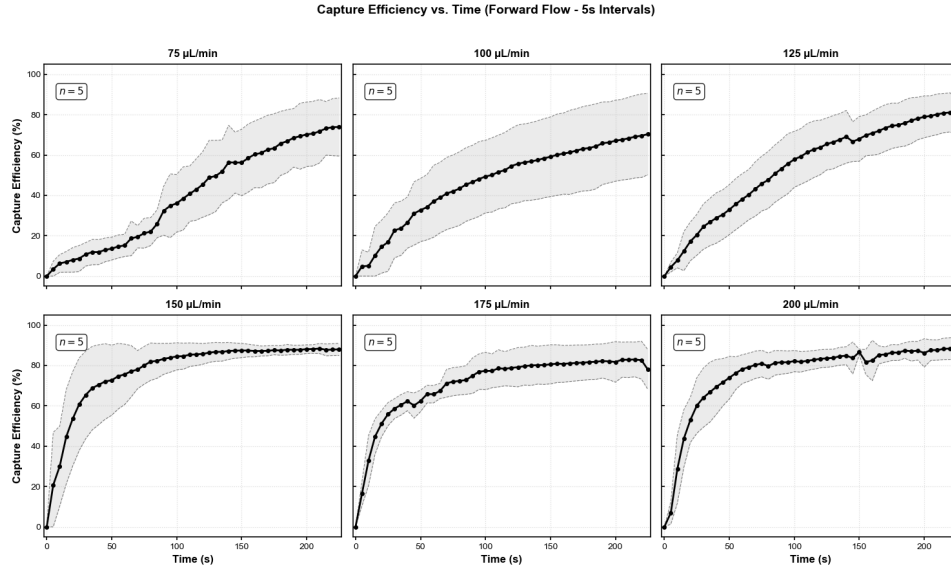


**Figure 2.5.** 10  $\mu\text{m}$  bead capture efficiency 130 seconds during reverse loading for flow rates between 75 and 200  $\mu\text{L}/\text{min}$ . The centerline is the mean and the gray dashed area is the standard deviation from it. Measurements were recorded in 5 second intervals.

Figure 2.6 shows bead loading in the forward direction across the identical range of flow rates. A similar shift from rapid asymptotic plateauing at elevated flow rates to a linear loading trajectory with diminished efficiency at lower rates was observed in forward loading. However, the maximum capture efficiency achieved across all operational flow rates was reduced compared to reverse flow, where rates below 200  $\mu\text{L}/\text{min}$  failed to consistently reach an average capture rate exceeding 80%. Nevertheless, a baseline capture rate exceeding 70% was sustained for flow rates above 100  $\mu\text{L}/\text{min}$ , indicating that the forward capture regions of the traps are similarly



capable of reliable particle acquisition and retention across a broad operating range.



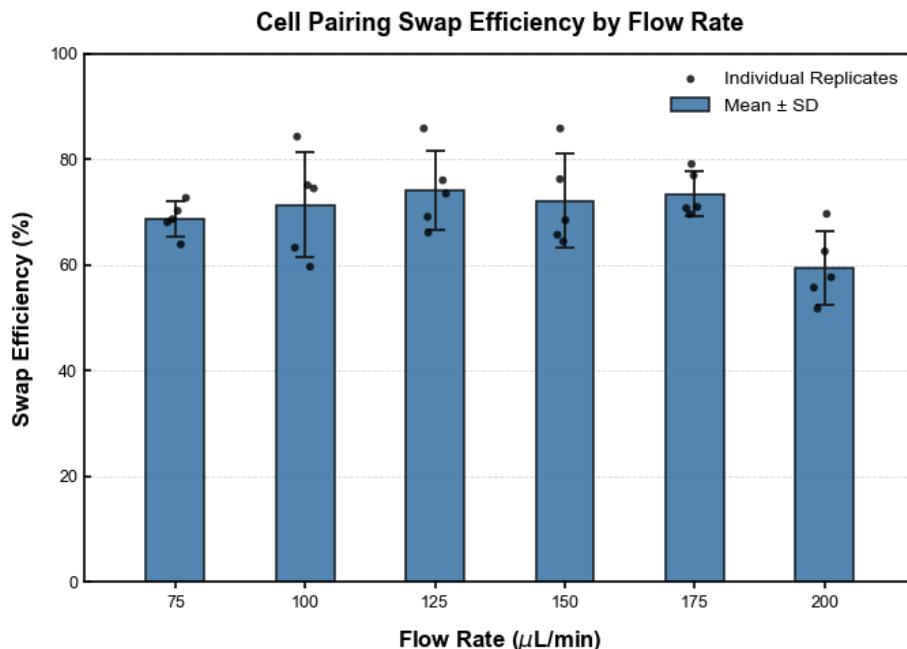
**Figure 2.6.** 20 μm bead capture efficiency 240 seconds during forward loading for flow rates between 75 and 200 μL/min. The centerline is the mean and the gray dashed area is the standard deviation from it. Measurements were recorded in 5 second intervals.

Figures 2.5 and ?? demonstrate a consistent trend wherein reverse loading progresses more rapidly than forward loading, and reduced flow rates yield lower trapping efficiency. Nevertheless, both loading phases consistently achieve high final occupancy at elevated flow rates, demonstrating the effectiveness of the trapping architecture even within a high-density, large-scale array.

## 2.5.2 Swap Efficiency

Figure 2.7 summarizes the measured swap efficiencies following flow reversal across the tested range of flow rates. The swap efficiency displayed a bell-shaped dependency on the applied flow rate, defining an optimal fluidic regime between 125 and 175 μL/min. Within this operational envelope, pairing yields remained robustly above 70%, with several replicates exceeding 80%. This wide optimal range demonstrates that the pairing mechanism is relatively insensitive to moderate variations in fluid velocity. However, performance decreased at the boundary flow rates, dropping to  $68.7 \pm 3.2\%$  at 75 μL/min and more markedly to  $59.5 \pm 6.9\%$

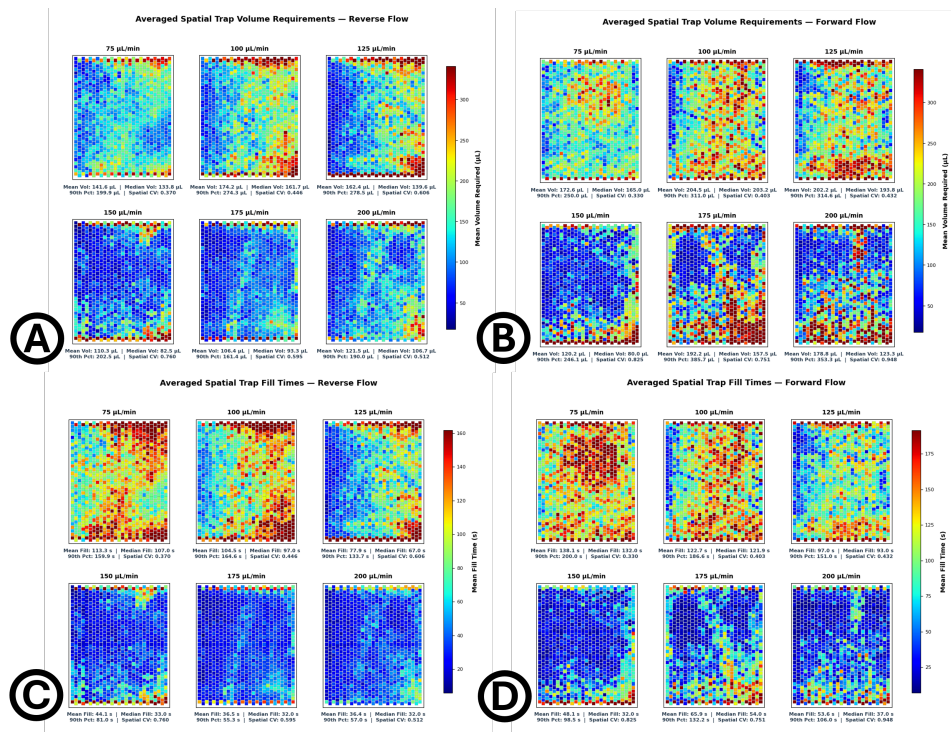
at 200  $\mu\text{L}/\text{min}$ . Consequently, operating within the 125 to 175  $\mu\text{L}/\text{min}$  window maximizes swap efficiency while allowing flexibility in fluidic driving conditions.



**Figure 2.7.** The efficiency with which the 10  $\mu\text{m}$  diameter beads swapped from the reverse end of one trap to the front end of another. The displayed flow rate for the swap was the same rate used for forward and reverse loading in the trap.

### 2.5.3 Spatial Occupancy

To better understand spatial loading dynamics throughout the trap array, the mean fill time and fluid volume required to populate each trap were calculated for a representative half-array (blue box in Figure 2.4) under forward and reverse flow conditions across all evaluated flow rates. Trap fill time served as a measure of temporal efficiency, whereas total fluid volume evaluated the reagent efficiency regarding bead solution usage. Required fluid volume was determined by multiplying local trap fill times by the volumetric flow rate, thereby decoupling capture efficacy from elapsed time and providing a flow-rate-independent metric to compare intrinsic trapping mechanics between conditions. This allows comparison of efficacy between flow rates better.



**Figure 2.8.** The analyzed region of the array is the blue boxed region in Figure 2.4. **A & B)** Volume taken to fill trap locations in reverse and forward directions respectively. **C & D)** Time taken to fill trap locations in reverse and forward directions respectively. The fluid flows left to right in all heatmaps.

Evaluating both temporal and volumetric loading efficiencies has shown utility in identifying optimal operational flow rates. As an example of the application of these two metrics, 150  $\mu\text{L}/\text{min}$  yields optimal performance in forward flow for minimized loading time (Figure 2.8D), whereas reverse flow achieves maximum speed at 175 and 200  $\mu\text{L}/\text{min}$  (Figure 2.8C). However, evaluating total required fluid volume reveals that while 150  $\mu\text{L}/\text{min}$  maintains superior reagent efficiency in forward flow (Figure 2.8B), the volume efficiency of reverse flow at 175 to 200  $\mu\text{L}/\text{min}$  drops below that of 150  $\mu\text{L}/\text{min}$  (Figure 2.8A). Thus, optimal flow conditions depend heavily on the target parameter, such as speed versus bead consumption, with 150  $\mu\text{L}/\text{min}$  offering the most balanced trade-off.

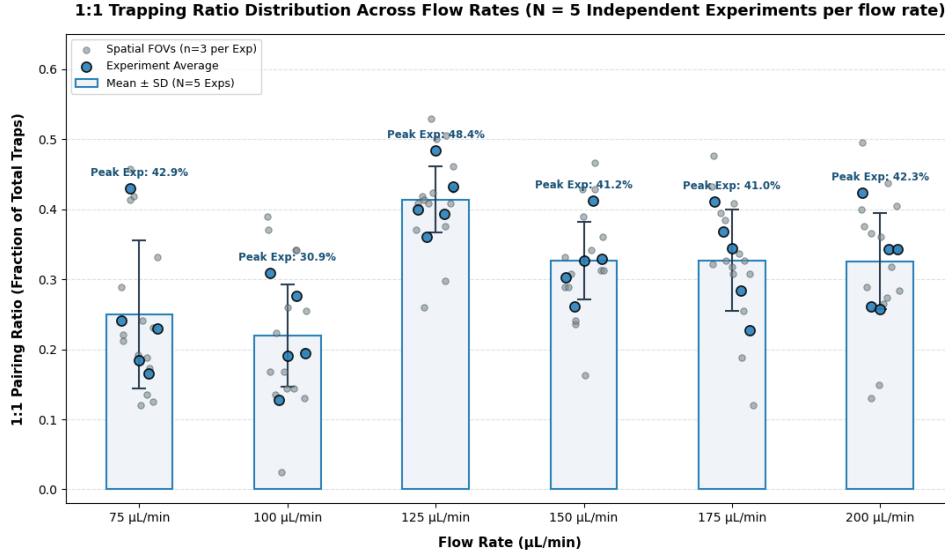
Loading behavior seen in Figure 2.8 is consistent with fluidic propagation along the path of least resistance, where flow initially funnels through the center of the channel and later deflects laterally as populated trap sites increase hydraulic resistance. As shown in Figure 2.3, COMSOL simulations suggest the reduced flow velocity around the traps following particle capture, leading to higher flow resistance. The absence of persistent unpopulated regions indicates that incoming microparticles were distributed with sufficient lateral uniformity to populate both the full width and length of the array.

#### **2.5.4 Bead Pairing**

Ultimately, the primary objective of the flow-reversal device was to achieve single-particle (1:1) pairs between two distinct particle populations. Figure 2.9 summarizes the distribution of heterogeneous particle pairings observed within the targeted sample regions following completion of sequential loading. The intended 1:1 pairings between 20  $\mu\text{m}$  (green) and 10  $\mu\text{m}$  (red) particles occurred most frequently at 125  $\mu\text{L}/\text{min}$ , yielding a mean pairing yield exceeding 40% and specific fields of view reaching up to 53% efficiency. Similar to the loading dynamics, pairing efficiency displayed a bell-curve relationship with flow rate, peaking at 125  $\mu\text{L}/\text{min}$  and sharply declining at both higher and more prominently at lower operational flow rates.

Individual gray data points represent single fields of view (FOVs) containing 208, with

three FOVs acquired per chip or experimental run. The three FOV data points collected within a single experiment were averaged to yield the blue data points, which serve as a representative estimate of overall performance across the entire trap array.



**Figure 2.9.** Percentage of 1:1, bead pairings observed in the analyzed fields of view. Bars represent the mean values, while whiskers represent one standard deviation. Blue data points represent independent experimental trials compiled by averaging three representative fields of view.

Together, these results demonstrate that the flow-reversal architecture enables efficient capture of two distinct bead populations during both forward and reverse loading phases. The hydrodynamic flow-reversal method for particle relocation was thus demonstrated as an effective mechanism for particle-pairing applications. Although the mean 1:1 pairing yield was approximately 40%, the high density of the 1,566 total trap array corresponds to an estimated average of 626 paired sites per chip based on the three sampled fields of view, substantially exceeding the absolute throughput of conventional microfluidic trapping architectures. This combination of high capacity and localized pairing fidelity suggests the flow-reversal platform as an appropriate architecture for single-particle interaction studies.

## 2.6 Conclusion

This work presents a single-layer, flow-reversal microfluidic device designed to advance the capabilities of hydrodynamic particle pairing for single-particle interaction studies. Utilizing a reversible flow architecture within a high-density, staggered trap array (1,566 sites), the platform enables the sequential capture, translocation, and 1:1 pairing of two distinctly sized particle populations. Fabricated using standard soft-lithography techniques, the device achieves high absolute capture yields without requiring complex multi-layer valve integration, providing an accessible, scalable, and efficient tool for high-throughput particle interaction assays.

Characterization of the flow-reversal device demonstrated successful particle capture, repositioning, and pairing within the highly dense trapping array. Evaluation of reverse and forward loading over time, swap efficiency, spatial trapping behavior, and bead pairing established the operational parameters governing device performance across diverse fluidic regimes. Because of peak performance occurring at different flow rates depending on the optimized parameter, this work provides insight into how to choose parameters according to preferred outcomes. The results establish the design as a promising approach for studies requiring controlled pairing of heterogeneous particle populations.

Future work will focus on establishing a non-dimensional scaling model to map the hydrodynamic behavior of synthetic microparticles to less rigid or density-variable cellular species, while refining fluidic protocols to minimize multiplet trap occupancy during translation to in vitro cell-pairing studies.

Chapter 2, in part is currently being prepared for submission for publication of the material. Friend, James; Salari-Namin, Sahand; and Croker, Ben, 2026. The dissertation author was the primary researcher and author of this material.

# Appendix

## A1 Fabrication Details

### A1.1 Master Mold Fabrication

Mold fabrication was performed at the Nano3 cleanroom facility at the University of California, San Diego using a 100 mm (4-inch) silicon substrate (University Wafer ID 452). The wafer was cleaned by submerging in acetone and sonicated for 5 minutes at medium intensity. The substrate was then drained and sequentially rinsed in an identical manner, performing two consecutive sonication cycles with isopropyl alcohol (IPA) and deionized (DI) water. The wafer was then blow dried with nitrogen gas and then heated at 100°C for 5 minutes to dehydrate it. Following dehydration, the surface was activated via oxygen plasma pretreatment (PE-100) at 250 Watts and 5 standard cubic centimeters (sccm) of oxygen for 5 minutes. Next, 4 mL of SU-8 3025 photoresist was dispensed onto the wafer surface by pipettes and spin-coated by ramping to 500 rpm at 100 rpm/s for 5 seconds, followed by ramping to 4000 rpm at 300 rpm/s before holding for an additional 25 seconds to achieve a photoresist layer thickness of 22  $\mu\text{m}$ . The coated wafer was then removed and extra photoresist on the bottom or sides was cleaned with acetone. The wafer was softbaked by placing it onto a hotplate at 65°C for 3 minutes followed by 95°C for 15 minutes. It was subsequently loaded into a direct-write maskless aligner (MLA 150, Heidelberg Instruments) and photolithographically patterned with the microfluidic trap architecture using a 375 nm laser source at a dosage of 650 mJ/cm<sup>2</sup>. Following exposure, a post-exposure bake was performed at 65°C for 1 min and 95°C for 5 min. The pattern was developed by immersing the substrate in SU-8 developer with continuous agitation for 8 min to dissolve unexposed photoresist. Residual uncrosslinked resist was cleared with a final rinse of fresh developer. After drying the wafer with nitrogen gas, feature quality was inspected via microscopy; if unexposed photoresist remained, development was resumed in 1-minute increments. Upon sufficient development and feature quality, a final hard bake step was conducted on the wafer as it is heated for 1 minute at 65°C, 1 minute at 95°C, and 5 minutes at 150°C. The mold is

then treated with an anti-sticking vapor (FDTS, Perfluorodecyltrichlorosilane) for 10 minutes, followed by 10 minutes of water to ensure proper feature detachment.

### **A1.2 PDMS Casting**

Next, the mold was cast with polydimethylsiloxane (PDMS, SYLGARD™ 184), as in standard soft lithographic protocols [32]. To facilitate release, the master mold was placed in an aluminum-foil-lined glass Petri dish. 50 grams of PDMS is then mixed using a 10:1 weight ratio of the base polymer to its curing agent. To mix the two components, a centrifugal mixer (ThinkyMixer, ARM-310) was operated at 2000 rpm for 3.5 minutes. The wafer was cleaned of surface particulates utilizing nitrogen gas prior to pouring liquid PDMS over the mold. The assembly was then placed in a vacuum desiccator to remove any trapped air under the PDMS. This degassing process was expedited by venting the desiccator chamber every 10 minutes to burst residual surface bubbles. After desiccation, remaining bubbles on the surface are removed with nitrogen gas applied perpendicular to the fluid surface. The PDMS was then thermally cured at 80°C overnight. After curing, the PDMS was carefully peeled off the silicon wafer, preventing direct physical contact with the feature side (the surface with the device pattern). The PDMS was then diced into individual devices before having their inlet and outlet holes made using a 0.5 mm diameter biopsy punch, driven from the feature side. To remove debris generated during punching, the feature side of the chip was cleared using a small section of adhesive tape applied gently across the inlets and outlets and peeled away. Glass slides were solvent-cleaned through the rinsing of the bonding surface with IPA and DI water, followed by drying using nitrogen gas. Both the PDMS devices and their respective glass slides were placed into a UV/ozone cleaning system (Novascan UV Ozone System) with bonding surfaces facing upward for a 5 minute exposure cycle. Immediately following surface activation, the pairs are taken out and gently pressed together to initiate bonding. Finally, the chip was baked for 30 minutes at 80°C to promote irreversible covalent bonding between the PDMS and the glass.



### A1.3 Bead Specifications

**Table 2.2.** Properties and specifications of the fluorescent microspheres used in the experiments.

| Parameter            | 10 $\mu\text{m}$ Red Beads                                     | 20 $\mu\text{m}$ Green Beads                    |
|----------------------|----------------------------------------------------------------|-------------------------------------------------|
| Bead Manufacturer    | Thermo Fisher Scientific / Molecular Probes                    | Polysciences, Inc.                              |
| Product Number       | Cat. No. F8834 (FluoSpheres Carboxylate-Modified Microspheres) | Cat. No. 18103-6 (Fluoresbrite YG Microspheres) |
| Material             | Polystyrene                                                    | Polystyrene                                     |
| Density              | 1.05 g cm <sup>-3</sup>                                        | 1.05 g cm <sup>-3</sup>                         |
| Surface Chemistry    | Carboxylate-modified                                           | Unmodified                                      |
| Fluorescence Channel | Cy5 ( <i>Ex/Em</i> = 625/645 nm)                               | GFP ( <i>Ex/Em</i> = 441/486 nm)                |
| Concentration        | 1.5 million beads per millilitre                               | 1.5 million beads per millilitre                |

## A2 Experimental Procedure

The microfluidic chip was connected to 0.5 mm ID, 1.5 mm OD Tygon tubing via 23 gauge steel pins and operated using a syringe pump (Pump 11 Pico Plus Elite, Harvard Apparatus). The tubing exiting the chip led to a dual diagonal 4-way valve, allowing routing of forward flow through the chip inlet to or outlet for flow reversal. To prevent bead aggregation, sonicated prior to injection into the chip. Before bead introduction, the chip was flushed with IPA at 150  $\mu\text{L}/\text{min}$  for 1 minute to remove air bubbles. To minimize dead volume, the bead suspension was pumped through the Tygon tubing to the terminal steel pin interface prior to insertion in the chip, allowing for near-instantaneous arrival time. The mixture of 10  $\mu\text{m}$  diameter fluorescent red beads and IPA are then flowed into the trap array from the reverse direction at flow rates ranging from 75-200  $\mu\text{L}/\text{min}$  until sufficient population. During this loading phase, the capture efficiency is recorded on the top half of the array. After sufficient population, IPA was flowed through the trap in the same direction to clear out excess beads at the same flow rate from 75-200  $\mu\text{L}/\text{min}$  in the reverse direction. After excess beads were cleared, still with constant perfusion at the same rate in the reverse direction, the diagonal 4-way valve was rapidly toggled to actuate an

instantaneous reversal of flow to the forward direction. Images of the whole trap array are then taken before the flow-reversal occurs for measuring both capture efficiency and flow-reversal efficiency. IPA is then flowed into the array at the same flow rate from 75-200  $\mu\text{L}/\text{min}$  for 2 minutes in the forward direction to relocate beads into their respective traps. Following the bead repositioning, another picture is taken of the whole array. Next, the 20  $\mu\text{m}$  diameter fluorescent green beads are flowed in the front at a flow rate ranging from 75-200  $\mu\text{L}/\text{min}$  in the forward direction until sufficient population while being recorded. An additional 2 minutes of IPA flow in the forward direction is used to remove excess beads. During final imaging, a constant 25  $\mu\text{L}/\text{min}$  flow rate in the forward direction is maintained to ensure beads remain at their capture sites. All images and videos except for the three close-up fields of view were captured using a Swiftcam 10 Megapixel camera with a 4x objective with an exposure time of 50 milliseconds and gain of 300%. The brightfield images taken of the fields of view with the 10x objective had an exposure time of 500 milliseconds and no gain. For the fluorescence imaging, GFP (green channel) and Cy5 (red channel) filters were used.

### **A3 Image Processing**

To conduct image processing and data analysis, custom Python scripts utilizing OpenCV and Matplotlib were developed to analyze acquired images and videos. For fields of view imaged under a 10 $\times$  objective in both brightfield and fluorescence channels, the brightfield channel was used to manually define trap boundaries, while two distinct color masks were applied in the hue, saturation, and value (HSV) space to segment the green and red fluorescent beads. The HSV threshold bounds were set to  $H \in [42, 61]$ ,  $S \in [190, 255]$ , and  $V \in [147, 255]$  for green beads, and  $H \in [0, 41]$ ,  $S \in [40, 255]$ , and  $V \in [168, 255]$  for red beads. Subsequently, minimum and maximum area thresholds were applied to the binary masks to isolate single microparticles. Automated trap counts were manually audited to verify pairing accuracy; image analysis was not performed blinded to experimental flow rates. A comparable segmentation workflow was implemented for full-array images acquired under a 4 $\times$  objective; however, this analysis omitted

color masks and instead relied on intensity thresholding to exploit the contrast between opaque microparticles and the brightfield background.

Traps with unresolvable boundaries or ambiguous fluorescence signals were excluded from analysis, as were uncaptured microparticles located outside the designated trap regions. However, all multiplet occupancies, clogged, incorrectly paired, or overpopulated traps were quantified and included in the final report with representative examples provided in Appendix A4.2.

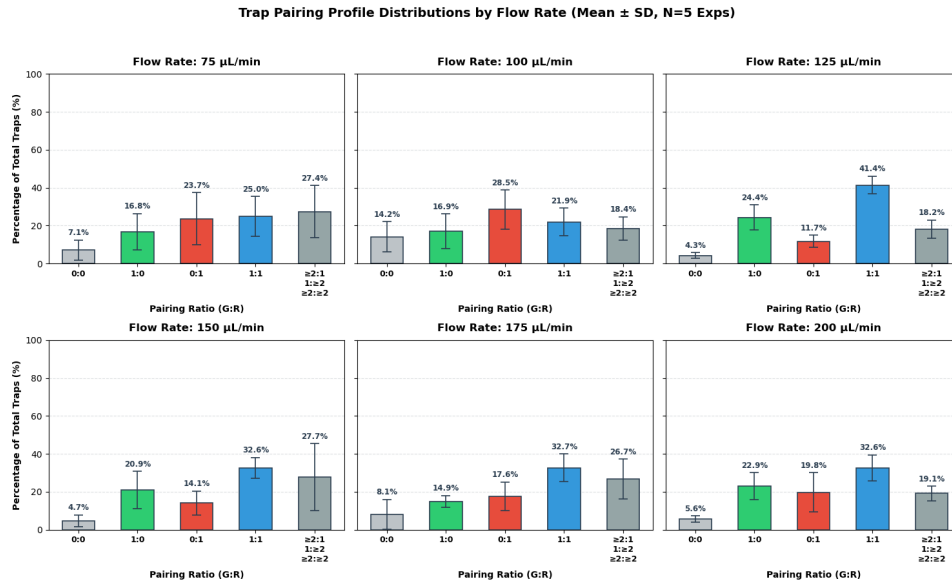
## A4 Supplementary Data and Figures

### A4.1 Endpoint Values

**Table 2.3.** Average capture and swap efficiencies across various flow rates.

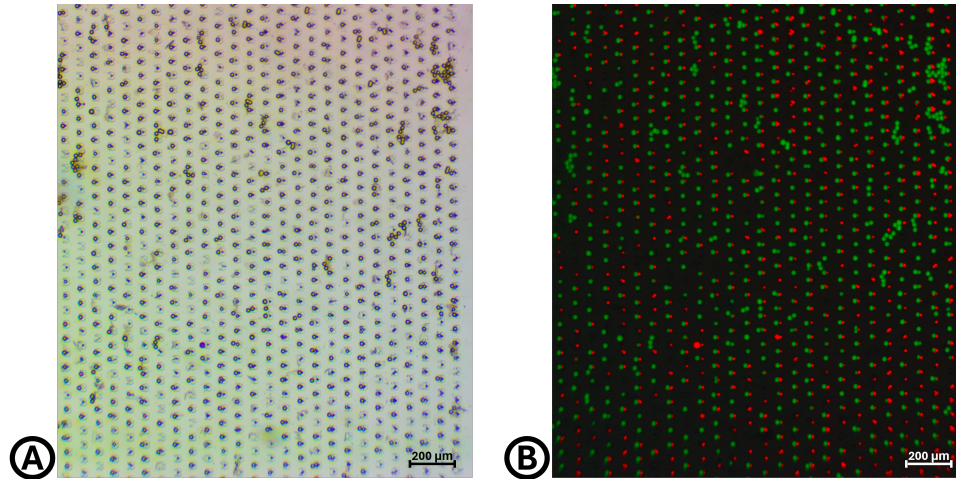
| Flow Rate                  | Final Reverse Capture | Final Forward Capture | Swap Efficiency  |
|----------------------------|-----------------------|-----------------------|------------------|
| 75 $\mu\text{L min}^{-1}$  | $65.6 \pm 14.2\%$     | $73.9 \pm 14.4\%$     | $68.7 \pm 3.2\%$ |
| 100 $\mu\text{L min}^{-1}$ | $74.7 \pm 11.8\%$     | $70.4 \pm 20.2\%$     | $71.4 \pm 9.9\%$ |
| 125 $\mu\text{L min}^{-1}$ | $84.5 \pm 7.6\%$      | $78.2 \pm 8.4\%$      | $74.2 \pm 7.6\%$ |
| 150 $\mu\text{L min}^{-1}$ | $90.8 \pm 4.5\%$      | $88.0 \pm 3.0\%$      | $72.2 \pm 8.9\%$ |
| 175 $\mu\text{L min}^{-1}$ | $95.5 \pm 1.0\%$      | $77.9 \pm 9.9\%$      | $73.5 \pm 4.3\%$ |
| 200 $\mu\text{L min}^{-1}$ | $94.1 \pm 4.2\%$      | $88.6 \pm 5.6\%$      | $59.5 \pm 6.9\%$ |

## A4.2 Pairing Ratios

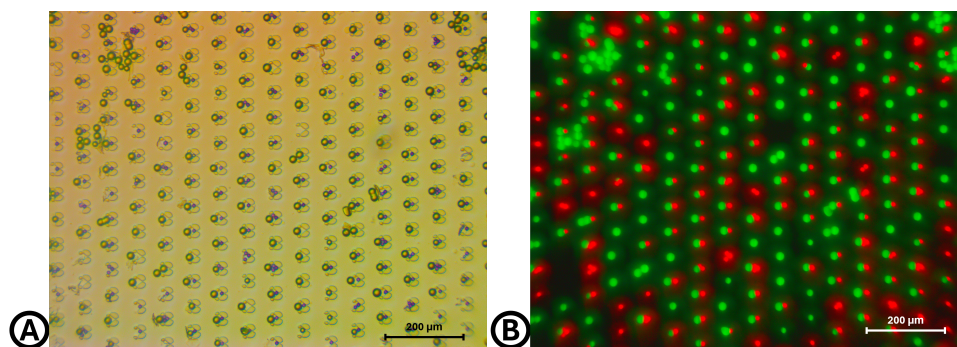


**Figure 2.10.** The bead pairing ratios of the different flow rates with their respective mean and standard deviation. Notable best performance on 125  $\mu\text{L}/\text{min}$ .

## A4.3 Fluorescence Images



**Figure 2.11.** A) Brightfield and B) fluorescence imaging of a trap array after loading, performed at 125  $\mu\text{L}/\text{min}$  under a 4x objective.



**Figure 2.12.** A) Brightfield and B) fluorescence imaging of a trap array after loading, performed at 150  $\mu\text{L}/\text{min}$  under a 10x objective.

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