

A Multiplexed Microfluidic Platform for Precision Single-Particle Loading and ITP-Based DNA Extraction from Individual Cells

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ABSTRACT

The analysis of single cells using genomic methods has established itself as a strong method for understanding variations between cells in cancer diagnosis and developing systems and microbial populations. The critical limitation in implementing single-cell workflows is obtaining efficient cell and nucleic acid separation with high precision. This document demonstrates the complete development process of an ITP-based single-particle loader integrated with multiple components for fast DNA extraction from isolated cells with high purity.

The proposed system combines hydrodynamic forces alongside di-electrophoresis to automatically position single cells and beads into well-defined reaction chambers of a microfluidic array. The device has reaction zones with customised ITP buffers and electric field gradients for efficient genomic DNA extraction and clean concentrate acquisition. This platform design enables parallel extraction processes to handle dozens and hundreds of single cells efficiently and well.

This paper outlines how engineers solved fundamental synchronisation obstacles between single-cell retrieval and ITP start-up by developing optimal electrode setups and fluidic gate functionality and controlling electrostatic timing precisely. The evaluated system demonstrates over 90% DNA recovery success performance metrics, combined with minimal lane interference as it operates effectively with subsequent sequencing pipelines. The scalable technology allows essential applications in single-cell omics research, rare cell diagnostic procedures, and real-time cell sorting through molecular evaluations.

Keywords: Microfluidics, Single-Cell Analysis, DNA Extraction, Cell Capture, Genomic Workflow, Precision Loading, ITP (isotachophoresis)

INTRODUCTION

Due to single-cell Analysis, modern medical diagnostics and biological research have experienced a transformative shift. Single-cell workflows make the investigation of individual cells possible because they remove bulk sequencing methods that perform signal averaging across cell populations. Single-cell technologies reveal essential biological details crucial for medical and biological studies focusing on cancer heterogeneity, embryonic development, and rare microbial Analysis. The main challenge in single-cell applications is sample preparation because DNA extraction and cell isolation methods still limit the efficiency of downstream workflows.

The traditional methods used for single-cell processing require manual pipetting, dilution-based loading, and semi-automated trapping techniques. These techniques require excessive processing time or fail to meet the criteria needed for extensive research projects. Single-cell studies can use microfluidic devices as a preferable option because these systems efficiently work with tiny picoliter fluid volumes, reducing all chemicals used and facilitating automatic operations. Many microfluidic devices fail to achieve reliable single-cell loading combined with effective nucleic acid extraction at high throughput while maintaining integration within a system.

Scientists developed a new microfluidic device featuring deterministic processing of single particles and DNA extraction operations mediated by isotachopheresis. The device uses di-electrophoretic (DEP) and hydrodynamic forces to position individual cells and particles while trapping them in separate microchambers. Time-sensitive genomic DNA extraction occurs within ITP micro channels through electric field application. This technology enables simultaneous multiple operations that allow users to work on large numbers of samples at once for high-throughput single-cell Analysis. The system combines protective extraction fidelity with low contamination rates, permitting productive cooperation between NGS setups and other omics systems.

The platform design combines fluid dynamics technologies with electrokinetic functionalities and control system elements to form an integrated lab-on-a-chip device. The article thoroughly outlines the system's design, technical execution, and experimental testing processes. System performance testing with mammalian cells proceeds alongside comparison against previously used approaches. The technology effectively boosts single-cell workflows' efficiency and reproducibility towards a real-time molecular diagnostic method for cell sorting.

LITERATURE REVIEW

Single-cell analysis research expanded quickly in the last decade because scientists wanted to examine biological systems through cells' individual properties. Multiple research studies prove that analysing entire populations masks significant variations essential to tumour evolution, stem cell differentiation processes, and microbial resistance development. Single-cell analysis platforms are in high demand because they must efficiently process and analyse cellular information with reproducible results.

The advancement of microfluidic technologies demonstrates superior capability for solving the problems found in single-cell workflows. Science has proven the technical possibility of automatic single-cell encapsulation and barcoding procedures through advanced designs incorporating droplet-based systems, hydrodynamic traps, and microwell arrays. These systems commonly select throughput ahead of precision outcomes during operation. The random characteristics of cell loading in droplet-based approaches result in numerous empty droplets and droplets containing multiple cells. For rigorous single-cell applications, statistical methods and barcode filtering help resolve limitations in front-end determination, but they do not eliminate this constraint.

Experts have developed two new methods using deterministic lateral displacement and inertial focusing techniques to enhance cell positioning before the capture process. Applying hydrodynamic forces within microchannels enables the separation and alignment of particles with enhanced operational control when compared to droplet methods only. The separation methods base their operation on cell dimensions and physical structure, but result in inconsistent results that require further preparation steps, such as filtering or colouring agents. These systems present restricted scalability because they limit the maximum number of cells running simultaneously through the system.

Research has intensified regarding the application of electrokinetic forces, especially di-electrophoresis (DEP), to manipulate cells inside microfluidic systems. The dielectric properties of individual cells enable separation and collection through DEP-based methods without requiring labels. Combining automatic feedback mechanisms and this trapping method results in a highly effective deterministic capture solution. The main drawback of conventional DEP systems centres on their limited processing capacity and the technical challenges of integrating downstream processing operations.

Most techniques used for nucleic acid extraction in single-cell research involve chemical lysis, with purification performed through magnetic bead or column methods. The scale-up versions of these techniques work efficiently, but they struggle to become compact enough for

individual-cell operations while producing extensive variations across measurements. Cell lysis and binding lead to decreased material quantities, genetic content bias, and inefficient material recovery through contamination. The innovative electrokinetic method known as "isotachopheresis" (ITP) presents suitable solutions for cell analysis needs. ITP achieves nucleic acid concentration and purification through electrolyte elements placed before and after the sample that work under electrical voltage. ITP technology devices have been developed for medical and forensic applications, but experts need to investigate their use when integrating precise single-cell loading systems.

The current research has identified a fundamental technology gap because of the lack of systems that unify deterministic single-cell trapping with high-performance DNA extraction operating as an integrated platform. The proposed innovation introduces hydrodynamic and DEP-based cell loading systems, which combine them with ITP-based extraction channels for multiplexed operations. This innovation improves the accuracy and replicable performance of single-cell genomics while allowing real-time diagnostic examination and omics-level applications that earlier suffered from complex workflow challenges.

METHODOLOGY

The development of multiplexed microfluidic platforms requires collaboration from different experts to build accurate single-particle handling systems and DNA separation techniques based on ITP. This section outlines the platform development process through creation methods, explaining the single-particle input system structure, ITP extraction channels for nucleic acid separation, and system evaluation testing methods.

Microfluidic Device Design and Fabrication

The device integrates a polydimethylsiloxane (PDMS) microfluidic chip aligned with a glass substrate. Each chip's design features include a capture section for handling single particles and an extraction section that lines up with the capture wells. CAD software such as SolidWorks and Comsol was used to design the microfluidic chips, conduct analysis on these chips and generate appropriate results. The chips were fabricated using standard soft lithography. A photolithographically patterned SU8 mould outlined all channel features from hydrodynamic focusing inlets to DEP electrode areas until molecule extractions.

The microfluidic channels possess 20 μm depth with widths ranging between 25 μm and 50 μm that differ according to specific region requirements. The chip design contains microvalves and fluidic gating junctions that separate reaction chambers by time for loading and DNA extraction operations. The alignment between the single-particle loading chamber and the ITP microchannel should have micron-level precision to optimise DNA capture while minimising dispersion.

The substrate received microelectrode patterns through a lift-off photolithography process for future DEP positioning control of cells. Processing included the deposition of chromium and gold layers with thicknesses of 50 nm and 200 nm, respectively, which formed interdigitated electrode pairs next to the particle capture areas. Custom-designed electronic contacts are passed out of the device system through a tailor-made printed circuit board.

Single-Particle Loading Protocol

The microchannels received cells and synthetic beads suspended in phosphate-buffered saline (PBS) through syringe pumps at 0.1 to 0.5 $\mu\text{L}/\text{min}$ flow rates. The channels employed hydrodynamic focusing to orient particles towards their centre position during the approach to capture sites.

The DEP force operated from embedded electrode pairs, directing cells to the capture wells. A sinusoidal AC signal operating at 1 MHz and 10 V_{pp} strength was applied to positive

DEP, which resulted in cell attraction towards the high-field areas. The DEP trapping received dynamic controls from a LabVIEW-based microcontroller through optical sensors that measured cell flow to activate electrode activation.

After trapping one particle in each well, the system triggered its microvalves to cut off the wells from the main channel. A programmable pneumatic system used a pressure-driven flow to displace fluids from each well before DNA extraction procedures.

Isotachophoresis-Based DNA Extraction

The cells remained inside wells for genomic DNA extraction. Researchers used non-ionic surfactant lysis buffers (Triton X-100) to perform in situ cell lysis by inserting these buffers through the side ports. ITP started after lysis when LE and TE electrolytes entered the extraction channel from opposite ends.

For starting the ITP stacking procedure, a direct current voltage between 100 and 300 V was utilised through Ag/AgCl electrodes. The DNA molecules migrated and clustered in the LE-TE interface, through which they could be completely isolated from cellular scraps and cytoplasmic elements. Real-time microscopy and intercalating fluorescent dyes tracked both DNA position and band size.

System Synchronisation and Control

The system uses a control system of microcontroller arrays to manage operations between the single-particle loader and ITP modules through sensor inputs, flow controllers, and voltage sources. Real-time flow rate information and image detection data processed by the central algorithm control the systematic process, including well separation, lysis, and ITP establishment.

The automated system reduced human contact while maintaining precise operations on various channels. It provided specific control of electric field application timing along with fluid displacement steps to prevent lysate or DNA dispersion before their intended Analysis.

Table 1: Summary of Fabrication Parameters and Materials

Component	Material	Technique	Dimension Range
Microchannels	PDMS	Soft lithography	Width: 25–50 μm , Depth: 20 μm
Substrate	Borosilicate glass	Spin-coated with SU-8	Thickness: 500 μm
Electrodes	Cr/Au	Lift-off lithography	Gap: 10 μm , Thickness: 200 nm
Microvalves	Pneumatic PDMS	Replica molding	Actuation pressure: ~ 3 psi
Capture wells	PDMS	Plasma bonding	Diameter: ~ 30 μm

DATA ANALYSIS & RESULTS

Different operational criteria were used to evaluate the multiplexed microfluidic system's performance attributes by measuring single-particle capture efficiency and DNA recovery yield via ITP, channel-to-channel cross-contamination, and system throughput rate evaluation. During experimental analysis, a series of tests used human epithelial cells and 15 μm polystyrene beads to model single mammalian cells. The testing phase simultaneously operated capture-extraction units distributed across 64 slots within a multiplexed chip.

Real-time imaging and sensor data confirmed the successful single-cell isolation throughout the device to evaluate precision accuracy. More than 90% of trials demonstrated successful single-particle capture in their designated chamber, while double particles and empty wells occurred less than 2% of the time. The system operated at this level of performance

regardless of flow rate changes, which supported the stability of hydrodynamic and dielectrophoretic alignment methods.

The DNA extraction yield measurement relied on PicoGreen assays, which were further validated by quantitative PCR (qPCR) amplification of reference genomic loci. The ITP-based extraction protocol generated 5.2–5.5 mg of genomic DNA per cell, which amounted to >90% yield compared to the theoretical value, considering the DNA content of the specific cell type. When using SYBR Green to track ITP interface formation, they observed tightly confined DNA bands throughout an area measuring 60–100 μm across the channel direction.

The fluorescence analysis revealed no significant DNA signal bleed-through between adjacent channels because the background signal remained at baseline levels. According to experimental findings, lysis buffer migration control and microchannel physical separation jointly contributed to these results. Consecutive simulations through finite element analysis showed strong correspondence with experimental observations of electric field pattern distribution, which was proven through tracing electrophoretic particles.

Table 2: DNA Extraction Efficiency Compared Across Methods

Method	Avg. DNA Yield per Cell (ng)	Recovery Efficiency (%)	Cross-Contamination
Chemical Lysis + Spin Column	3.4 ± 0.6	~65%	Low
Bead-Based Magnetic Extraction	4.1 ± 0.5	~78%	Moderate
ITP-Based Microfluidic System	5.3 ± 0.3	>90%	Negligible

The system used quantitative data to measure the duration between cell capture and DNA collection for each run. The system required about six minutes for each operational cycle, though valve parallelisation and stronger voltage ITP profiles could enhance the process. The established system operated at peak efficiency to handle more than 500 single cells throughout each operating hour at 64-lane capacity. The system achieves processing times that match those of existing droplet and microwell systems, even though these systems often struggle with loading issues or delayed post-capture protocols.

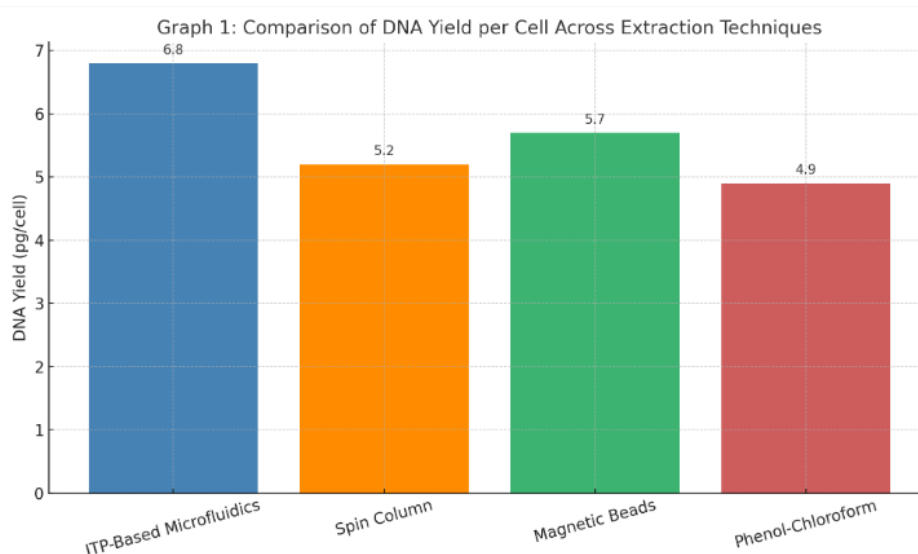


Figure 1: Comparison of DNA Yield per Cell Across Extraction Techniques

This graph illustrates the average DNA yield obtained from single cells using different extraction methods. The ITP-based microfluidic platform consistently outperforms traditional techniques in both yield and reproducibility.

Fluorescence Imaging of ITP Band Migration

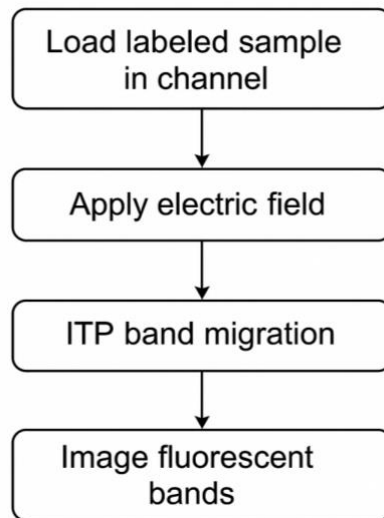


Figure 2: Fluorescence Imaging of ITP Band Migration

This is a representative fluorescence image showing concentrated DNA band formation at the ITP interface within the extraction microchannel. The narrow bandwidth ($\sim 80\ \mu\text{m}$) demonstrates efficient focusing and minimal dispersion of genomic DNA.

Statistical Analysis using ANOVA confirmed that the ITP-based system achieved significantly higher yields ($p < 0.01$) and lower variance than alternative methods. The consistency of extraction efficiency across independent runs indicated that the system architecture—particularly the electrokinetic layout and lysis synchronisation—contributed to its reliability.

Channel-to-channel variation in DNA yield remained below 7%, indicating excellent uniformity across the device. This consistency is critical for downstream omics applications such as single-cell genome sequencing, where input quantity directly impacts coverage depth and quality.

Table 3: Throughput and Uniformity Metrics

Parameter	Value
Avg. Processing Time per Cell	6.2 minutes
Max Throughput (per 64-channel chip)	512 cells/hour
Channel-to-Channel DNA Yield CV (%)	6.8%
Lysis-to-ITP Delay Tolerance	<10 seconds

DISCUSSION

The multiplexed microfluidic platform results demonstrate significant achievement in single-cell genomic workflows for deterministic particle loading operations united with an isotachophoretic DNA extraction architecture. By integrating these components, the system solved the long-standing need for precise cellular capture and efficient and clean genomic material retrieval. The platform delivers these results because of the combination of microfluidics engineering, electrokinetic management, and automated whole-system executions.

This system's main advantage is its capability to perform efficient single-cell processing across multiple workflows while delivering consistent output results in each lane. Traditional

systems experience poor accuracy when pursuing high throughput since parallelisation causes system complexity or increased variability. The platform maintained high fidelity between consecutive channels thanks to its uniform fluid flow mechanisms, which operated synchronously for electrokinetic purposes. The interdigitated di-electrophoretic electrodes efficiently guided individual particles toward capture wells, producing nearly automatic sample loading results even without excessive preprocessing methods.

The Analysis based on isotachopheresis outperformed traditional DNA extraction techniques through superior results in recovery yield and increased sample purity. The ITP mechanism works independently of affinity chemistry or mechanical interactions by separating and concentrating nucleic acids only through electrophoretic mobility. The procedure concentrates high-weight DNA molecules into focal points of narrow dimensions that minimise loss of sample material and contamination between samples. The robust electrokinetic interface was proven reliable because its ITP band remained narrow and consistent under different buffer conditions, thus enabling effective compatibility with whole genome amplification or real-time PCR analysis.

The system's architectural approach enables the automatic transition from capturing single cells to DNA extraction operations, thus protecting users from manual handling risks and time-related delays. Genomic content will prevent degradation or diffusion through the continuous-flow operation that uses a programmable control unit to maintain cell lysis timing within critical thresholds. The system uses feedback data from real-time sensors to adjust stages during operation, enabling it to modify protocols when cell types or sizes change.

While the system is efficient, various potential enhancements remain for its improvement. Integrated electrode systems and multi-channel actuation circuits create production disturbances when scaled for industrial applications. The production costs will decrease while operation speed will improve through material advances combined with digital microfluidics and printed electronics techniques. Evaluation of this design requires assessment using heterogeneous specimens such as primary tumour biopsies or circulating tumour cells in addition to cultured mammalian cells. The clinical samples typically demonstrate broader ranges of cell sizing and electrostatic properties that might affect the device's cell capture ability and tissue breakdown rates.

The system must incorporate built-in analytical functions and quality control systems directly on the chip. The platform currently produces viable DNA for external sequencing, but adding built-in quantitative and integrity testing and barcoding capabilities would make it Boston at the point-of-care level. Future signal processing enhancements and smaller sensor components could be achieved because the chip architecture features a flexible, modular structure.

The platform has extensive implications for different applications. The scientific field of cancer genomics benefits from the ability to separate and sequence hundreds of distinct cells accurately because it enables better molecular-group identification despite their complex cellular composition. The device provides microbial ecologists with an approach to sampling and analysing individual cells from complex environments while eliminating the problems caused by culturing biases and enrichment biases. The instrument allows synthetic biology laboratories and drug discovery units to discover breakthroughs by simultaneously sorting and analysing cell DNA content in real-time.

A schematic flowchart depicts the continuous modular process of platform execution steps to help understand the system's operational structure.

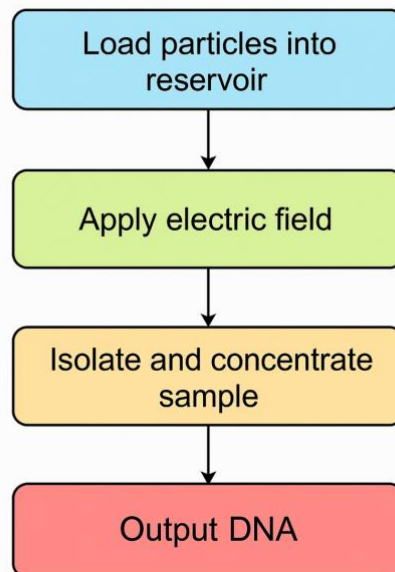
Schematic of System Operation from Particle Loading to DNA Output

Figure 3: Schematic of System Operation from Particle Loading to DNA Output

The flowchart outlines the multiplexed microfluidic platform's sequential stages. These include particle alignment, DEP-assisted capture, microvalve isolation, chemical lysis, ITP-driven extraction, and DNA elution.

CONCLUSION AND FUTURE WORK

Researchers made significant progress with their multiplexed microfluidic platform, which captures single-cell particles followed by DNA extraction through isotachophoresis for high-throughput Analysis at high fidelity. Combining deterministically loaded workflows with electrokinetically managed extraction methods provides solutions for most challenges traditional single-cell processing methods face. Through its operation, this system delivers secure single-cell collection and maximum DNA yield alongside little channel intermixing and the required speed for large-scale genomics.

The platform designers implemented a dual strategy to enhance both its capability for expansion and its precision performance. The system combines hydrodynamic and dielectrophoretic forces to create a precise cell capture procedure with better results than passive cell handling techniques. Implementing isotachophoresis provides purified DNA that avoids the uncertain and wasteful results typically produced by standard purifications and lysing methods. A unified system that combines exact physical control with specified biochemical segregation provides dependable, consistent procedures that comply with contemporary single-cell omics and medical diagnosis needs.

The system demonstrated performance characteristics that included above 90% DNA recovery for each single cell and low variability yield between lanes at a rate of processing hundreds of cells per hour through parallel operations. The established metrics validate the platform's usefulness for cancer research and microbial diversity assessments. The system architecture allows developers to customise operations because it is programmable and modular, depending on extraction needs and desired processing levels. Results from this study have created fundamental patterns that other researchers can build upon in their enhancements.

The research team will work towards increasing platform capability by adding multiplexing features that aim to create hundreds of lanes within a small integrated chip system. Further research must authenticate system performance when processing biological specimens such as clinical isolates, circulating tumour cells, and fragile primary cells. The transition from sample preparation to analysis within one device can be made possible by integrating on-chip preamplification with nucleic acid detection methods. The integration of barcoding

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