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Transforming microfluidics for single-cell analysis with robotics and artificial intelligence

Jinxiong Cheng, †[ab] Rajiv Anne†[ab] and Yu-Chih Chen *[abcd]

Single-cell analysis has advanced biomedical research by revealing cellular heterogeneity with unprecedented resolution, identifying rare subpopulations that drive disease progression and therapeutic resistance. Microfluidics is central to this advancement, enabling precise single-cell isolation, manipulation, and cellular profiling. However, limitations in automation, reliability, and technical barriers hinder the widespread adoption of microfluidic single-cell analysis. This review highlights key innovations in experimental methods and deep learning-driven data analysis to overcome these challenges. Operating microfluidics with robotic operation, digital microfluidics, or microrobots enhances experimental precision and scalability. Beyond experimental automation, deep learning revolutionizes data interpretation through label-free image processing and cell status classification and regression. Generative models further refine analysis by correcting batch effects and generating synthetic datasets, improving accuracy and reproducibility in single-cell studies. Considering the complexity of integrating these technologies, remote shared cloud labs represent a potential pathway toward standardized and high-throughput single-cell analysis, facilitating broader access to advanced experimental workflows. Overall, the convergence of robotics and artificial intelligence in single-cell analysis will change data acquisition, hypothesis testing, and model refinement, driving breakthroughs in drug discovery and personalized medicine. While implementation remains challenging, this paradigm shift is transforming biomedical research, enabling unprecedented precision, scalability, and data-driven innovation.

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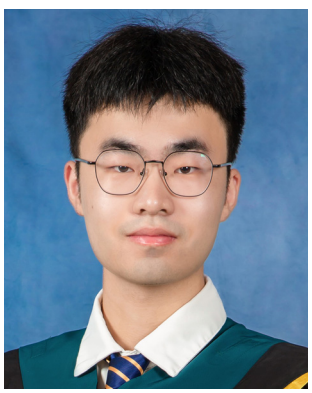
1. Introduction

1.1. Historical convergence of microfluidics, robotics, and AI

Over the past two decades, microfluidics, robotics, and artificial intelligence (AI) have each undergone remarkable technological evolution, individually transforming the landscape of biological research. When viewed through a historical lens, these three fields reveal a converging trajectory that now positions their integration as both inevitable and impactful, especially in the realm of single-cell analysis. As illustrated in Fig. 1, the microfluidics field emerged with the advent of polydimethylsiloxane (PDMS) soft lithography[1] and Quake valves[2] in the early 2000s, enabling precise fluid control over cellular microenvironments.[3,4] Digital microfluidics (DMF) subsequently introduced enhanced automation and programmability, with droplet volumes exhibiting coefficients of variation (CVs) as low as 1%, thereby enabling reliable single-cell manipulation.[5–7] Droplet microfluidics[8] enabled massively parallel single-cell

[a] *Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, 3700 O'Hara Street, Pittsburgh, PA 15260, USA*
[b] *UPMC Hillman Cancer Center, University of Pittsburgh, 5115 Centre Ave, Pittsburgh, PA 15232, USA. E-mail: cheny25@upmc.edu; Tel: +1 412 623 7701*
[c] *Department of Computational and Systems Biology, University of Pittsburgh, 3420 Forbes Avenue, Pittsburgh, PA 15260, USA*
[d] *CMU-Pitt Ph.D. Program in Computational Biology, University of Pittsburgh, 3420 Forbes Avenue, Pittsburgh, PA 15260, USA*
† Equal contributions.



Jinxiong Cheng

Jinxiong Cheng is a Ph.D. student in the Department of Bioengineering at the University of Pittsburgh, working in Dr. Yu-Chih Chen's laboratory. He received his B.S. degree in Biomedical Engineering from the Southern University of Science and Technology (SUSTech), Shenzhen, in 2023 under the mentorship of Prof. Xingyu Jiang. During his senior year, he conducted his undergraduate thesis research in Prof. Aaron Wheeler's laboratory at the University of Toronto, focusing on digital microfluidics. His current research interests center on high-throughput microfluidic technologies and their applications in cancer biology.

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encapsulation (~44 000 single cells in a single run) and laid the foundation for technologies like Drop-seq[9] and CITE-seq.[10] However, its implementation often involves complex fluidic control systems and surfactant management. Organ-on-a-chip platforms improved biological relevance and disease modeling fidelity through biomimetic architectures yet often lacked the spatial resolution and throughput necessary for robust single-cell-level analysis, such as spatial heterogeneity profiling.[11–14] In parallel, robotics progressed from basic mobile platforms like AIBO[15] and Roomba[16] to sensor-rich collaborative robots (*e.g.*, PR2 (ref. 17)), and eventually to human-assistive or autonomous systems capable of delicate biological operations. This trajectory has enabled robust sample handling, fluidic actuation, and physical automation of complex workflows, essential in biology. Meanwhile, the rise of deep learning and large language models transformed AI from static classifiers to adaptable, multi-modal systems capable of interpreting imaging, text, and command instructions. AI-guided decision-making was being integrated into real-time optimization and context-aware control in healthcare applications.[18] The synergy among these domains is no longer aspirational but has become technically and conceptually aligned. In the context of single-cell research, microfluidics provides the physical interface for cell handling, robotics enables scalable and precise execution, and AI supplies the intelligence to drive adaptive control and data interpretation. This convergence is essential not just for scaling experiments, but for enabling new modes of inquiry in precision biology and clinical diagnostics.

With the advancement of robotics, the concept of a "robot scientist" was first introduced by King *et al.* in 2004.[19] They envisioned an automated system capable of both hypothesis generation and experimental execution, integrating machine learning to acquire background knowledge and analyze results. Building on this vision, Soldatova *et al.* developed ontology of scientific experiments (EXPO) in 2006, the first formal ontology to digitally capture and structure every aspect of the scientific process.[20] This foundation enabled the creation of *Adam*, a robot scientist that autonomously generated biological hypotheses and performed wet-lab experiments to test them, particularly in yeast functional genomics.[21] Following this, the same group introduced *Eve*, a second-generation robot scientist focused on drug discovery.[22] Eve combined machine learning-based activity prediction with high-throughput screening to prioritize validation of candidate compounds, an early attempt to close the loop between *in silico* prediction and wet-lab verification. In parallel, Savall *et al.* developed an automated platform for Drosophila neuroscience that integrated robotic handling, microsurgery, machine vision, and behavioral analysis into a single pipeline.[23] During this period, platforms including Transcriptic (now Strateos) and Emerald Cloud Lab began offering remote-controlled, automated experimentation, which have been described as early demonstrations of the envisioned "cloud lab" concept. A significant breakthrough came with *Maholo*, a dual-arm humanoid robot introduced by Yachie *et al.* in 2017.[24,25] Unlike traditional liquid-handling robots confined to custom workstations, *Maholo*'s human-like architecture allowed it to operate standard lab tools without modification. This human-compatible design greatly enhanced its flexibility, enabling seamless integration into existing workflows and broadening its utility across diverse biological protocols. Since 2020, continued advances in robotics and AI have driven the

Rajiv Anne

Rajiv Anne completed his undergraduate studies in Bioengineering with a concentration in Cellular Engineering at the University of Pittsburgh, where he worked in Dr. Yu-Chih Chen's laboratory for three years. He also participated in the Summer Undergraduate Research Internship (SURI) at Pitt. He will be pursuing his master's degree at the Technical University of Munich. His research expertise centers on applying convolutional neural networks (CNNs) for label-free cellular morphological analysis.

Yu-Chih Chen

Yu-Chih Chen earned dual bachelor's degrees in Electrical Engineering and Law from National Taiwan University (2008) and a Ph.D. in Electrical & Computer Engineering from the University of Michigan, Ann Arbor (2014). He is an Assistant Professor in the Department of Computational and Systems Biology and at the UPMC Hillman Cancer Center, University of Pittsburgh. Dr. Chen has been recognized with the TSMC Outstanding Student Research Award and named an Emerging Forbes Scholar by the Forbes Institute for Cancer Discovery and Hillman Early Career Fellow. His research focuses on high-throughput single-cell analysis, microfluidics, and deep learning for cancer precision medicine.

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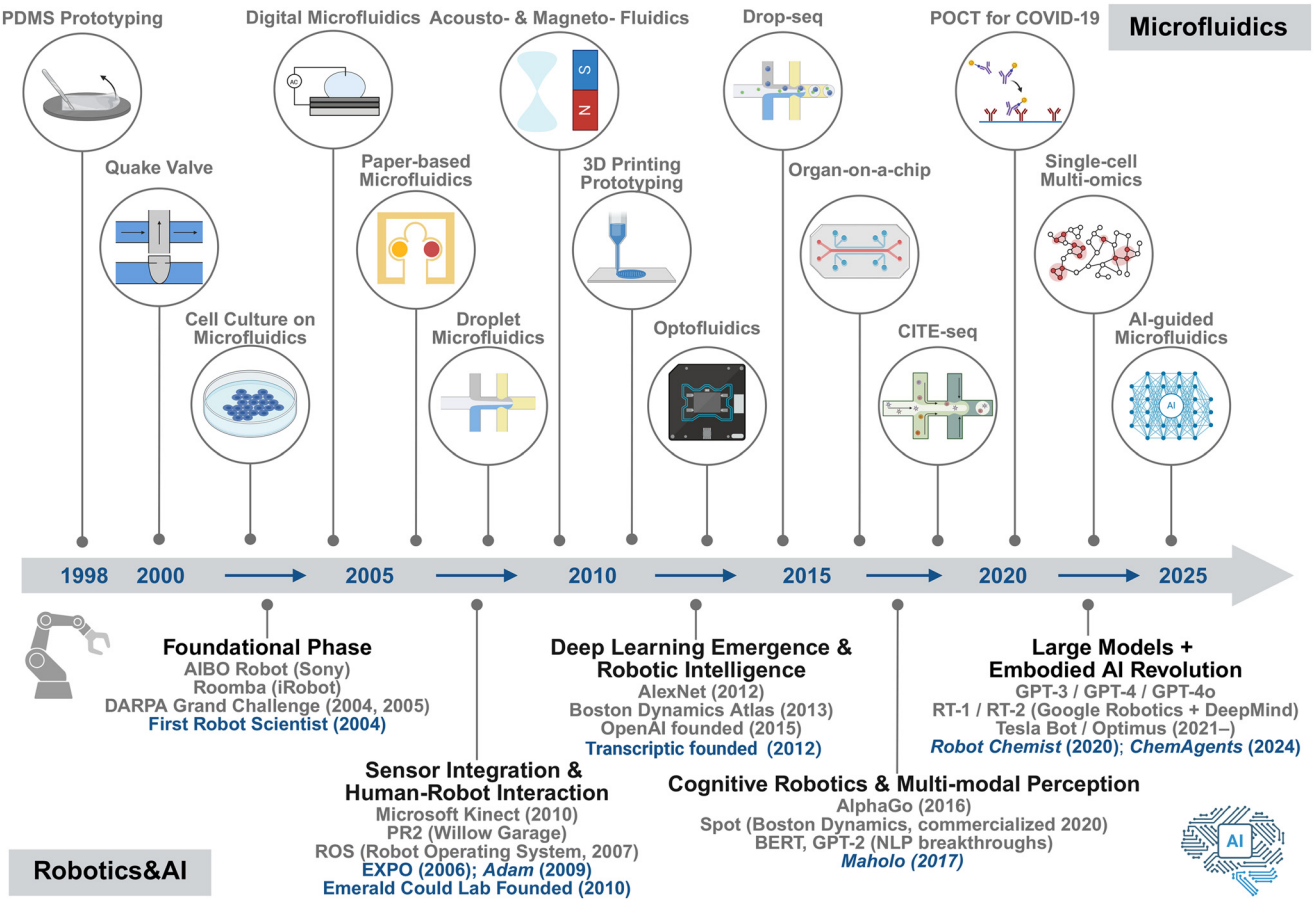

Fig. 1 Milestones in microfluidics, robotics and AI in two decades. This timeline highlights key milestones in microfluidics, from PDMS prototyping[1] to the emergence of AI-guided microfluidics.[187] Notable advances include the Quake valve,[2] cell culture integration,[4] digital,[6] paper-based,[188] and droplet microfluidics,[9] along with acoustofluidics,[189] magnetofluidics,[190] 3D printing,[191] and optofluidics.[192] Recent breakthroughs encompass Drop-seq,[9] organ-on-a-chip,[11–14] CITE-seq,[10] POCT for COVID-19,[193] and single-cell multi-omics.[95] Parallel progress in robotics and AI includes early consumer robotics (AIBO,[15] Roomba[16]), autonomous navigation through the DARPA Grand Challenge, and interactive systems such as PR2, ROS,[17] and Kinect.[194] The rise of deep learning with AlexNet[195] and achievements like AlphaGo[196] and GPT-2/3/4 (ref. 197) enabled smarter, perception-driven automation. By the 2020s, the introduction of RT-2 (ref. 198) and Tesla Optimus marked a shift toward intelligent robotic workflows. Key robotic scientist milestones include EXPO (2006),[20] Adam (2009),[21] Maholo (2017),[24,25] Robot Chemist (2020),[199] and ChemAgents (2024).[28] Created using BioRender, based on references.[1,4,6,9–17,20,21,24,25,28,95,187–199]

emergence of more intelligent and autonomous robotic scientists. For example, Cooper *et al.* developed a mobile chemist that employed Bayesian optimization to iteratively guide experiments.[26] The platform developed by Lunt *et al.* introduced coordinated multi-robot systems with autonomous decision-making,[27] while the ChemAgents system proposed by Song *et al.* leveraged large language models (LLMs) to perform six complex, multi-step experimental tasks.[28] While early implementations have largely centered on chemical research, recent advances in biological interfacing and intelligent control signal a growing opportunity within the life sciences, particularly in the context of single-cell analysis. In this review, we aim to explore the potential of such trio-interaction by examining how microfluidics provides the physical interface, robotics delivers precise and scalable execution, and AI enables adaptive decision-making. By synthesizing progress across these three domains, we seek to identify opportunities, challenges, and future directions toward truly intelligent biological studies.

1.2. Single-cell analysis in biomedical research

Single-cell analysis has emerged as a transformative approach in biomedical research, enabling the investigation of cellular heterogeneity with unprecedented resolution.[29–34] Unlike bulk analysis, which averages signals across a population of cells, single-cell techniques dissect the molecular and functional diversity within seemingly homogeneous tissues. This is particularly critical given that cellular heterogeneity is a fundamental property of biological systems, arising from genetic, epigenetic, and environmental variations.[35–38] Even within a clonal population, stochastic gene expression, differential microenvironmental interactions, and dynamic cellular states contribute to variability in function and fate. Traditional bulk measurements often obscure these crucial

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differences, masking rare but biologically significant subpopulations. By providing a high-dimensional view of cellular heterogeneity, single-cell analysis allows researchers to deconvolute complex biological processes, identify rare or transient cell states (<1%), and construct precise maps of tissue organization and disease progression.[39–41]

The impact of single-cell analysis is particularly evident in cancer and immunology research, where cellular heterogeneity directly influences disease outcomes. In oncology, single-cell transcriptomics has uncovered distinct tumor subpopulations with varying drug sensitivities, providing critical insights into therapeutic resistance.[42–46] Even a small fraction of drug-resistant cells, can drive disease progression and treatment failure.[47,48] Single-cell RNA sequencing (scRNA-seq) has identified stem-like subclones in high-grade serous ovarian cancer, fueling recurrence and metastasis.[49,50] Likewise, single-cell epigenomic profiling has revealed non-genetic mechanisms of drug tolerance, exposing adaptive resistance strategies that evade conventional detection.[51–54] Single-cell analysis has transformed immunology by revealing the functional diversity of immune cells, particularly within the tumor microenvironment. Even rare immune subsets, sometimes comprising less than 1–2% of the infiltrating population, can drive potent and divergent immune responses.[55–57] Tumor-infiltrating lymphocytes (TILs) exhibit highly heterogeneous activation states, with exhausted $CD8^+$ T-cell subsets constraining anti-tumor immunity.[58–60] Single-cell technologies have mapped distinct exhaustion trajectories, guiding strategies to optimize immunotherapy. Beyond cancer, single-cell profiling of peripheral immune responses in infectious diseases has identified novel immune signatures predictive of disease severity, advancing precision diagnostics and treatment.[61,62] The integration of spatial and multi-omics approaches is further reshaping biomedical research by uncovering deeper mechanistic insights, with current platforms achieving pixel sizes of 10–55 μm (capturing from single cells to small clusters) and enabling thousands of spatially resolved spots per tissue section.[63–65] By dissecting cellular heterogeneity, single-cell analysis is driving precision medicine, enabling patient-specific therapies based on single-cell molecular profiles rather than bulk tissue properties.

1.3. Microfluidics for single-cell analysis

Microfluidics which precisely manipulate and control fluids at the microscale level is one of the most important technologies in single-cell analysis.[66–68] This technology integrates principles from engineering, physics, chemistry, and biology to create miniaturized systems capable of performing various analytical, diagnostic, and biological assays. By leveraging the unique physical phenomena that occur at the microscale, such as laminar flow, surface tension, and capillary action, microfluidic devices enable the efficient handling of small volumes (picoliter–nanoliter) of liquids with high precision and throughput.[69,70] In the realm of single-cell analysis, microfluidics offers several key advantages that make it an ideal platform for studying individual cells at high resolution. First, microfluidic devices enable the isolation and manipulation of single cells with unparalleled precision (70–90%), allowing researchers to study cellular behavior and responses in a controlled microenvironment.[33,71,72] By confining cells within microchambers or droplets, microfluidic systems minimize cell-to-cell interactions and ensure uniform experimental conditions, enhancing the reproducibility and reliability of single-cell experiments. High-density microchambers or droplets drive parallel, high-throughput single-cell analysis, enabling rapid and precise large-scale cellular profiling (10^4–10^6 cells per run).[73] Integrating microfluidics with imaging, sensors, and molecular analysis techniques, such as fluorescence microscopy, RNA sequencing, and proteomic profiling, unlocks comprehensive, high-dimensional insights from individual cells at an unprecedented scale.[9,74–76] This capability is essential for characterizing cell-to-cell variability, identifying rare cell populations, and elucidating complex biological processes at the single-cell level. Moreover, microfluidic systems offer precise spatial and temporal control over cellular microenvironments, enabling researchers to mimic physiological conditions and study dynamic cellular processes in real time.[77–79] By modulating fluid flow, chemical gradients, and environmental cues within microfluidic devices, researchers can investigate diverse aspects of cellular physiology, including cell migration, proliferation, differentiation, and signaling dynamics, with high spatiotemporal resolution.

In addition to facilitating functional assays and dynamic cell behavior studies, microfluidics has also profoundly advanced the field of single-cell omics by enabling the precise manipulation and processing of individual cells across multiple molecular dimensions. In single-cell genomics, Zahn *et al.* developed a microfluidic device comprising 192 nanoliter-scale chambers to perform direct library preparation (DLP) without the need for preamplification, enabling efficient whole-genome sequencing of hundreds of single cells.[80] Building upon this approach, Laks *et al.* further introduced DLP+, an automated and scalable platform that integrates image-based single-cell identification and positioning, ultimately achieving processing of 51 926 single-cell genomes across diverse sample types.[81] Zheng *et al.* developed Microbe-seq, a droplet-based microfluidic platform that enables high-throughput single-microbe whole-genome sequencing by encapsulating individual microbes into droplets, followed by cell lysis, whole-genome amplification, and droplet-specific barcoding to achieve strain-level resolution in complex microbiomes.[82] In transcriptomics, Bai *et al.* developed the dielectrophoresis (DEP)-trapping-nanowell-transfer (DEP-dTNT) platform, which utilizes electric field-driven DEP to actively trap single cells into nanowells, followed by hydrodynamic co-loading of barcoded beads.[83] The paired cells and beads are then encapsulated within reaction

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chambers, enabling efficient mRNA capture and barcoding for downstream single-cell RNA sequencing. In proteomics, Zhu *et al.* developed the nanoPOTS (nanodroplet processing in one pot for trace samples) platform,[84] which enables highly sensitive proteomic analysis within reaction volumes smaller than 200 nL, achieving the identification of ~1500 to ~3000 proteins from as few as ~10 to 140 cells. Separately, Gebreyesus *et al.* introduced the single-cell integrated proteomic microfluidic chip (SciProChip),[85] a fully integrated system that automates single-cell isolation, counting, imaging, and sample preparation on-chip, and is directly compatible with data-independent acquisition (DIA) mass spectrometry for high-throughput single-cell proteomics. More recently, DMF platforms such as active-matrix digital microfluidic chip for single-cell proteomics (AM-DMF-SCP)[86] have demonstrated robust single-cell proteomic capabilities by enabling automated nanoliter-scale processing and label-free analysis, while addressing challenges of integration and sensitivity through active matrix control. In metabolomics analysis, researchers have developed approaches for intact single-cell metabolite profiling[87–89] or targeted metabolite extraction followed by mass spectrometry (MS) analysis.[90,91] More recently, microfluidic impedance cytometry has enabled one-step sample preparation for single-cell MS (>99% sorting/desalting, high purity), streamlining workflow and opening opportunities for multi-modal (electrical and metabolic) single-cell characterization.[92] In lipidomics, microfluidic platforms coupled with mass spectrometry (*e.g.*, MALDI-MS[93] and DASEI-MS[94]) have enabled single-cell lipid profiling with high structural resolution. In addition, the digital microfluidic isolation of single cells for -omics (DISCO) platform[95] leverages DMF in combination with AI-guided image processing and laser-based cell lysis to enable selective isolation and multi-omics analysis of individual cells, including their genomes, transcriptomes, and proteomes. Its tissue-resolved variant, tissue-DISCO (tDISCO),[96] extends this capability to spatially contextualized cells within tissue slices, providing an integrated view of molecular profiles with spatial precision. For more detailed discussions on microfluidics-based omics strategies, readers are referred to recent reviews by Gebreyesus *et al.*[97] and Zhang *et al.*,[98] which comprehensively summarize microfluidic innovations in single-cell proteomics and multi-omics integration, respectively.

While microfluidics has profoundly advanced single-cell omics by enabling precise spatiotemporal control and high-throughput cellular profiling, its broader implementation outside of specialized bioengineering laboratories remains constrained by several practical limitations. The fabrication of PDMS-based devices often requires several hours to a full day of casting, aligning, bonding, and punching, that require specialized equipment, cleanroom access, and technical proficiency. These devices are frequently single-use and prone to failure, and the process is susceptible to chip-to-chip variability that can compromise reproducibility. Even after fabrication, fluid control systems (*e.g.*, syringe pumps or pneumatic valves) and imaging or multi-omics integration require meticulous calibration and synchronization. Moreover, microfluidic experiments typically produce large, complex datasets, such as time-lapse microscopy or high-content omics, which demand sophisticated analytical pipelines and expertise in computational biology. These combined factors present significant barriers for many biology-focused labs. In this review, we not only discuss these limitations in detail but also highlight emerging solutions aimed at advancing and disseminating microfluidic technologies, such as standardized and automated platforms and data analysis tools, to make single-cell microfluidics more accessible, reproducible, and scalable.

2. Automation of microfluidics with robotics

Automating microfluidics with robotic operation seems straightforward, yet significant technical challenges remain. Microfluidic devices are predominantly fabricated using soft lithography with PDMS due to its biocompatibility, optical transparency, and gas permeability.[99,100] The fabrication process involves molding PDMS on a photolithographically patterned master, curing, and manually peeling and punching to create inlets and outlets. These manual steps introduce device-device variability, compromising reproducibility and posing challenges for robotic operation. Moreover, microfluidic systems depend on tubing for precise fluid control and seamless integration with various instruments.[101,102] While tubing provides adaptability, its connection and disconnection remain non-trivial for robotic handling, introducing additional complexity to fully automate workflows. Addressing these challenges requires advancements in standardized fabrication techniques and robotic dexterity for seamless microfluidic integration. Alternatively, non-PDMS microfluidic systems actuated by electrical or optical signals offer a promising solution to overcome the fabrication and operation challenges associated with PDMS-based platforms. Emerging digital microfluidics or microrobotic technologies have demonstrated initial success in achieving automation, yet there are still limitations in technical complexity, reliability, throughput, and scalability. In this section, we will highlight single-cell analysis advancements enabled by robotics.

2.1. Customized robotic systems for single-cell experiments

Despite existing challenges, several pioneering groups have developed customized robotic systems specifically designed for single-cell manipulation. As mentioned earlier, the nanoPOTS platform was built on a home-assembled robotic system comprising a high-precision syringe pump, a high-density nanowell-array chip with a droplet storage frame, an *x*–*y*–*z* translational stage, and a stereomicroscope (Fig. 2A).[84,103–105] Building on this foundation, an automated, label-free nanoproteomics imaging approach was introduced to

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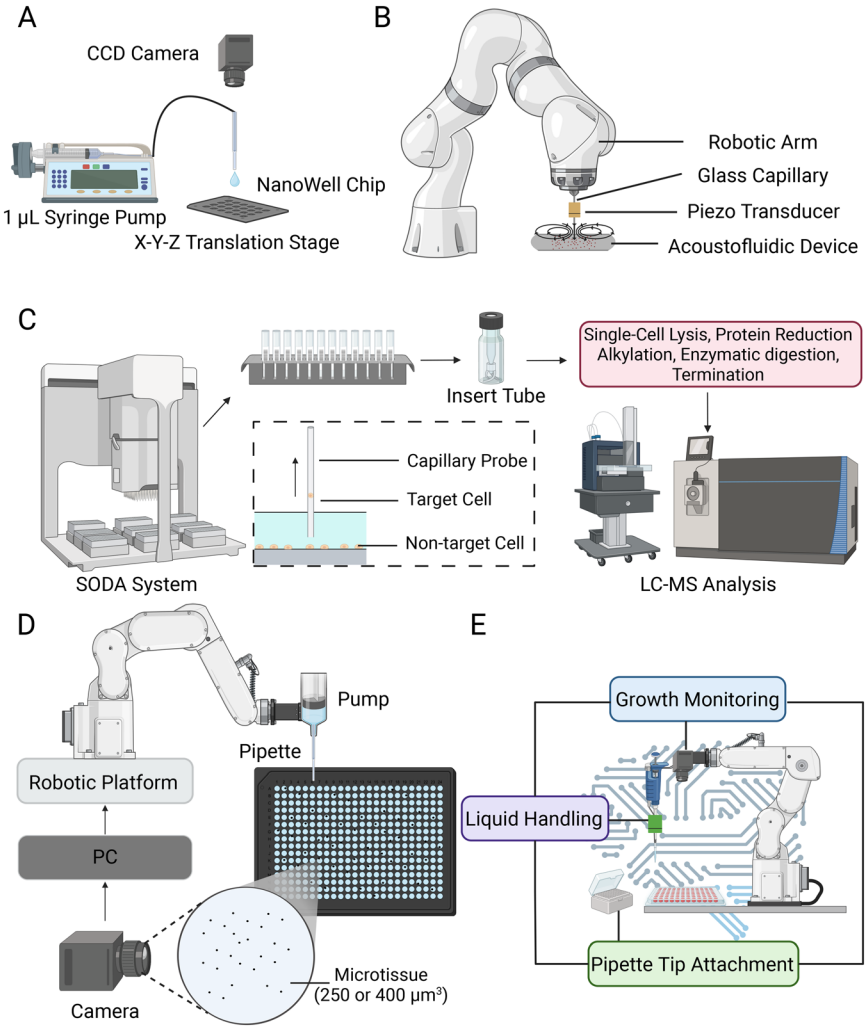

Fig. 2 Lab-built robotic systems for microfluidic operations in single-cell and microscale biological analysis. (A) The nanoPOTS platform integrates a high-precision syringe pump, nanowell chip, droplet capture frame, and stereomicroscope for picoliter-scale liquid dispensing in single-cell proteomics workflows.[84] (B) A robot-assisted acoustofluidic end effector system, integrating a robotic arm, glass capillary, piezo transducer, and acoustofluidic device to enable high-precision fluid mixing, particle manipulation, and biological sample handling.[107] (C) The PiSPA (pick-up single-cell proteomics analysis) system couples a capillary probe with a droplet-array-based liquid handling platform (SODA: sequential operation droplet array)[105] to isolate and process individual cells through sequential *in situ* lysis, digestion, and LC–MS injection.[108] (D) A compact robotic platform combining a pipette, syringe pump, onboard camera, and motion control system to automate microtissue handling in 384-well plates under sterile culture hood conditions.[110] (E) The RoboCulture system for yeast cultivation integrates real-time optical feedback and force sensing with robotic tip exchange and liquid handling to enable fully autonomous, long-duration culture and monitoring.[111] Created using BioRender, based on references.[84,105,107,108,110,111]

quantitatively map over 2000 proteins across mouse uterine tissue sections at a spatial resolution of 100 µm, enabling high-throughput, cell-type-specific spatial proteome analysis.[106] Durrer *et al.* introduced the robot-assisted acoustofluidic end effector (RAEE) system,[107] which merges robotics with acoustofluidics to overcome limitations in microscale liquid manipulation (Fig. 2B). Traditional lab processes rely on macroscale liquid handling, while lab-on-a-chip (LoC) technologies, despite their promise, often lack automation and adaptability. The RAEE system bridges this gap by integrating a robotic arm with an acoustofluidic end effector, enabling precise fluid pumping, particle trapping, embryo handling, and automated mixing of viscous liquids. Wang *et al.* developed the pick-up single-cell proteomic analysis (PiSPA) workflow,[108] a probe-based single-cell proteomic platform built on the sequential operation droplet array (SODA) liquid handling system[109] to enable nanoliter-scale cell capture, pretreatment, and liquid chromatography–mass spectrometer (LC–MS) injection (Fig. 2C). Target cells are aspirated individually and processed in insert tubes through *in situ* lysis, digestion, and other steps, minimizing sample loss and maximizing protein recovery. This workflow achieves deep proteome coverage, up to 3000 proteins per cell, and reveals cellular heterogeneity, as demonstrated in migrating HeLa cells.

While not all robotic systems were originally designed for single-cell analysis, several recent innovations in robotic

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microfluidic handling demonstrate a clear trajectory toward clinical relevance and intelligent operation in the field. For example, Stepanov *et al.* developed a compact, low-cost robotic platform integrating a pipette, syringe pump, and onboard camera to manipulate live microtissues in 384-well plates (Fig. 2D).[110] This system operates within standard tissue culture hoods without the need for pneumatic controllers or external microscopes, streamlining sterile workflows and enabling drug testing on limited patient biopsy samples. In another example, Angers *et al.* demonstrated RoboCulture, a fully automated platform for yeast cultivation that leverages real-time optical and mechanical feedback to guide experimental decisions over a continuous 15 hour protocol (Fig. 2E).[111] While the system remains limited in throughput (5 seconds per tip change) and format (96-well plate), it exemplifies an emerging paradigm in robotic microfluidics, one where vision systems, real-time inference, and robotic learning converge to support fully autonomous biological experimentation. These precedents, though not tailored to single-cell analysis, underscore the feasibility and importance of combining robotics and AI to overcome the manual bottlenecks of microfluidic operation and pave the way for next-generation automated biological research.

2.2. Integration of commercial liquid-handling robotics with microfluidic single-cell systems

In addition to lab-built robotic systems, numerous commercialized robotic liquid handlers have been applied in the microfluidics field. Owing to their throughput and reliability, many of these systems are designed for well plate formats, such as 3D spheroid cultures using hanging drop arrays.[112] For organ-on-a-chip applications, several research groups have developed their own robotic liquid handling platforms, for example, Novak *et al.* implemented a system for perfusing multiple vascularized organ chips,[113] while Jiang *et al.* designed a robotic workflow for organoid-based assays.[114] These platforms have significantly enhanced the throughput of tissue engineering applications and enabled the analysis of inter-organoid or inter-spheroid heterogeneity. However, they generally do not achieve single-cell resolution, as such drug screening applications often prioritize scalability over cellular-level precision.

To further extend the applicability of commercial robotic handlers in single-cell microfluidic systems, system-level optimizations are essential. One strategy involves encapsulating individual cells within water-in-oil droplets, facilitating precise reagent manipulation while preserving single-cell integrity and minimizing cross-contamination. Tran *et al.* demonstrated the automation of droplet microfluidics using commercial fluid-handling robotics (Freedom EVO, Tecan). Their approach, robotic operation of droplet microfluidics (RAD microfluidics), integrates key microfluidic components, such as droplet generators, mergers, and sorters, into fully automated workflows, significantly improving efficiency and scalability (Fig. 3A).[115] Alternatively, employing high-precision robotic platforms such as cellenONE, which supports picoliter-to-nanoliter dispensing and integrated image-based cell selection, can

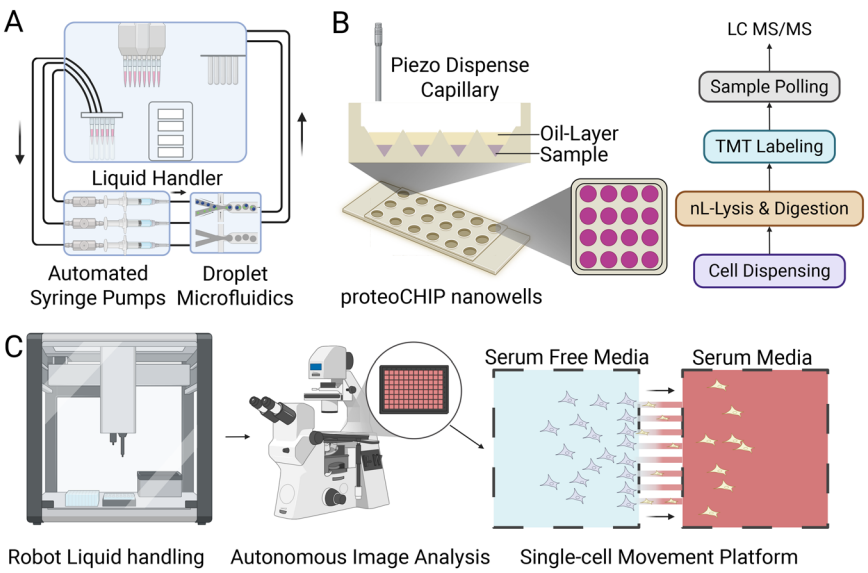

Fig. 3 Applications of commercial liquid-handling robots in microfluidic single-cell analysis. (A) A droplet microfluidics system coupled with a commercial liquid handling robot (Freedom EVO, Tecan) and automated syringe pumps.[115] (B) A nanoliter-scale single-cell proteomics workflow based on the proteoCHIP platform, integrated with the cellenONE robotic system. Single cells are dispensed into nanowells, followed by nanoliter-scale (nL) cell lysis and tryptic digestion, tandem mass tag (TMT) labeling for multiplexed quantification, and sample pooling for liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis.[116] (C) A high-throughput single-cell migration platform capable of tracking tens of thousands of individual cells. The system combines a liquid handling robot (OT-2, Opentrons) with autonomous image analysis software for microfluidic single-cell analysis.[117] Created using BioRender, based on references.[115–117]

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improve compatibility with low-volume microfluidic environments. Ctortecka *et al.* developed an automated platform for multiplexed single-cell proteomics using a custom-designed microfabricated chip (proteoCHIP) coupled with the high-precision robotic system cellenONE. Their streamlined workflow achieves exceptional sensitivity with minimal sample loss (Fig. 3B).[116] By eliminating manual handling and carrier proteome dependence, the platform enabled the identification of ~2600 proteins across 170 single cells, with high reproducibility and over 90% data completeness per run. Another approach is to engineer microfluidic devices with structures such as confined migration channels or single-cell traps to ensure controlled cell passage, thereby enhancing throughput and automation across single-cell workflows. Zhou *et al.* developed a high-throughput microfluidic platform capable of tracking single-cell movement under multiple treatment conditions in a 384-well format, fully compatible with robotic liquid handling (Opentrons, OT-2 Liquid Handler) (Fig. 3C).[117–119] This system incorporates autonomous image analysis software for real-time quantification of cell migration, encompassing image registration, quality control, channel segmentation, cell identification, and migration distance calculation in a semi-automated fashion requiring reduced human intervention, primarily for tasks such as plate handling, reagent loading, and experimental setup. Their innovative approach enabled the screening of 172 compounds for migration inhibition and toxicity, identifying promising low-toxicity regulators. With single-cell resolution, the system quantified both average migration distances and the behavior of top-ranked fast-moving cells, offering unparalleled insights into cell dynamics.

Despite technical challenges in integrating microfluidic single-cell analysis with robotic operation, this convergence holds the potential to enhance throughput, reliability, and reproducibility. Standardization in microfluidic fabrication is crucial, with a shift toward precision-engineered components, such as those produced *via* injection molding, replacing labor-intensive manual soft lithography processes. Simultaneously, advancements in robotics will enable increasingly intelligent systems capable of precisely handling complex fluidic networks. While obstacles persist, the rapid evolution of both microfluidics and robotics is steadily bridging the gap.

2.3. On-chip micro-robots for sample manipulation

Beyond robotic operation of microfluidics, advances in micro-robotics now enable precise, programmable single-cell manipulation. Among these, electromagnetic field-based

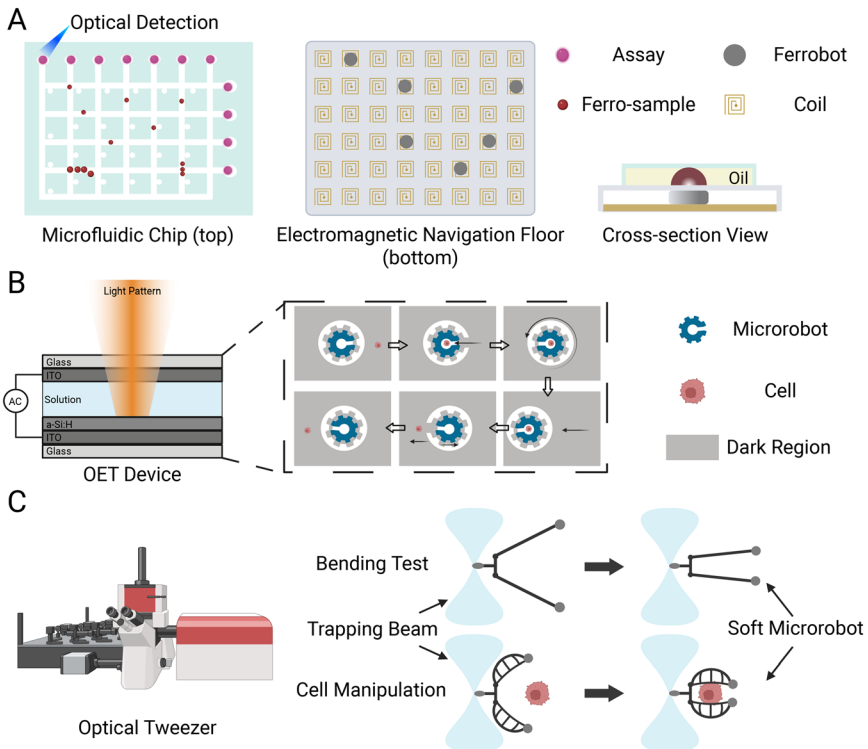

Fig. 4 On-chip microrobots for single-cell manipulation and analysis. (A) A digital microfluidic ferrobotics system enables high-throughput, programmable fluidic operations for biomedical diagnostics, including SARS-CoV-2 amplification and detection.[120,121] (B) An optoelectronic microrobot within an OET system achieves precise single-cell manipulation with minimal cellular damage. Light-controlled actuation allows targeted cell collection and transfer.[124] (C) Optically actuated, TPP-fabricated soft microrobots enable gentle, high-precision single-cell manipulation using optical tweezers. The designs illustrate deformation under trapping beam-induced forces, leveraging wireframe structures for controlled cell manipulation.[125] Created using BioRender, based on references.[120,121,124,125]

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control has emerged as a leading strategy, offering high-speed, programmable, and reproducible fluidic operations with robust performance. Yu *et al.* developed a ferrobotic system using electrowetting-on-dielectric (EWOD) to achieve parallelized and sequential droplet control in digital microfluidics, drawing inspiration from automated guided vehicles.[120] Lin *et al.* further enhanced this system by introducing a circuit board-based programmable platform, significantly improving SARS-CoV-2 amplification and detection efficiency (Fig. 4A).[121] In digital microfluidics, on-chip ferrobots not only preserve but enhance the advantages of low sample consumption and high throughput, establishing an efficient, high-fidelity operational platform for point-of-care and other biomedical applications.

In addition to ferrobotic systems, Chiou *et al.* pioneered optoelectronic tweezer (OET), leveraging image-based DEP to modulate photoconductive materials and precisely manipulate particles and cells with an electric field.[122] Zhang *et al.* advanced this concept with patterned OET (p-OET), integrating a continuous photoconductive layer to eliminate the need for constant illumination.[123] This breakthrough enabled the development of optoelectronic micro-robots, achieving programmable, submillimeter-scale manipulation (Fig. 4B).[124] These micro-robots generate larger, more uniform forces, minimize cellular damage, and offer flexible single-cell isolation for downstream applications such as clonal expansion and RNA sequencing.

Further expanding the capabilities of micro-robotic systems, soft micro-robots have emerged as an innovative approach for precise and stable control of cellular orientation under minimal forces (ranging from tens to hundreds of piconewtons). Soft micro-robots excel in collecting, sorting, transporting, and rotating individual cells with high sensitivity. Iványi *et al.* developed an elastic and deformable micro-robot using multiphoton polymerization, a technique that offers nanometer-scale resolution while minimizing biological damage (Fig. 4C).[125] This micro-robot is capable of performing tasks such as collection, rotation, release, and pairing of single cells. Notably, its actuation mechanism is based on optical tweezers (OTs), where traction, pushing, pulling, and deformation forces are generated by two-photon polymerization (TPP)-fabricated bendable nanorods or torsion nanostrings. Compared to opto-thermal manipulation, this non-contact OT-based approach minimizes thermal effects on cells while allowing vertical cell positioning. Experimentally, these soft micro-robots have demonstrated successful operation within microfluidic chambers, significantly enhancing the sensitivity and precision of single-cell manipulation.

2.4. Combination of robotic single-cell operation with deep learning

Based on robotic single-cell operation, there are some pilot studies including deep learning in the pipeline. Jin *et al.* presents a label-free live-cell imaging method that leverages deep learning to predict cell phenotypes based on whole-transcriptome sequencing.[126] This approach, enabled by the live imaging and cell picking system (ALPS), establishes a direct link between cell images and transcriptomic profiles, allowing real-time molecular characterization without compromising cell viability. This noninvasive and unbiased technique offers significant advantages for studying cell dynamics and functional heterogeneity. In parallel, Guo *et al.* introduced an artificial intelligence-assisted digital microfluidic framework (μDropAI) for multistate droplet control, which integrates semantic segmentation to recognize droplet morphology and interactions with an error rate $<0.63\%$.[127] By reducing the coefficient of variation of split droplet volumes to 2.74%, μDropAI improves precision in droplet manipulation and highlights the potential of semantic-driven DMF systems for fully automated and adaptive operation. Another breakthrough is a deep learning-based single-cell sorting platform developed by Guo *et al.*,[128] which integrates YOLOv8, a real-time object detection system, with digital microfluidics to achieve highly efficient and pure (>96%) single-cell isolation. Unlike conventional fluorescence-activated (FACS) and magnetic-activated (MACS) cell sorting, this label-free approach enhances precision (98.5% accuracy) by utilizing safe interval path planning (SIPP) for optimized droplet trajectory control. Together, these advancements highlight the transformative potential of deep learning in noninvasive single-cell analysis and high-precision cell sorting.

2.5. Commercial platforms for single-cell analysis

In addition to academic publications (Table 1), new commercial systems provide solutions in single-cell workflows (Table 2). Despite their diversity, these systems share a common goal: to enhance precision, reproducibility, and throughput in single-cell assays. These platforms utilize core microfluidic principles, digital, droplet-based, optofluidic, or capillary-driven, to enable diverse omics workflows, including single-cell RNA sequencing, multi-omics integration, and high-sensitivity proteomics. High-throughput systems such as BOXmini SCP (ACX Instruments), Chromium (10× Genomics) and Beacon (Bruker) exemplify DMF, droplet and optofluidic solutions for multi-parameter profiling. Other platforms, such as CellenONE, F.SIGHT, Cell Handler, and DispenCell, are designed primarily for single-cell isolation and dispensing and operate in well-plate formats. These systems integrate imaging and AI-driven decision, making them well-suited for workflows that require precise cell selection and deposition into multi-well formats. Their compatibility with standard labware and omics workflows makes them practical tools for screening and targeted single-cell experiments. While most robotic liquid handling systems rely on physical pipette contact to aspirate and dispense fluids, raising concerns about cross-contamination and shear-induced cell damage, non-contact strategies have

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Table 1 Comparison of robotic-microfluidic platforms for automated cell and omics analysis

Methods	Robotics	Omics/phenotypes	Principle	Sample type	Advantages	Limitations	Throughput	Ref
nanoPOTs	Custom *XYZ* robotic microdispenser system	Proteomics	Nanowell	Cells	Extremely low sample loss; high sensitivity	Requires custom-built platform	Low	84
RAEE	Dorna robotics arm	Cell manipulation	Acoustic streaming	Cells, particles	High-precision, label-free particle/cell manipulation; contactless operation	High system complexity; low throughput	Low	107
PiSPA	Self-built SODA system	Proteomics	Droplet	Cells	High proteome coverage; precise single-cell capture	Low throughput; robotic setup required	Low	105, 108
Live microtissue manipulation	Dobot MG400 robotic arm	Drug response	Liquid handling	Micro dissected tissues	Low cost and compact; high accuracy	Not for single cell	Medium	110
RoboCulture	Franka Emika robot, Robotiq 2F-85 gripper	Cell growth	Cell culture	Yeast cells	Fully autonomous closed-loop system; real-time decision-making; compatible with standard labware	Limited to specific cell types; not applicable to single-cell resolution	Low–medium	111
RAD microfluidics	Freedom EVO, Tecan	Genomics; other omics with modifications	Droplet	Cells	No user intervention theoretically	Requires custom valve/pump interfacing hardware	High	115
proteoCHIP	CellenONE®	Proteomics	Nanowell	Cells	Low sample loss, scalable, fully automated proteomics workflow	Endpoint analysis only; no cell recovery	Medium–high	116
Single cell migration assay	OT-2, Opentrons	Cell migration	Channel-based microfluidics	Cells, drug/compounds	High efficiency; compatible with imaging; semi-automated	Difficult to retrieve individual cells; limited compatibility with multi-omics analysis	Medium–high	117
Ferrobot	Ferrofluid-driven magnetic microrobots	MMP enzyme activity assay, nucleic acid detection	DMF	Human plasma, saliva	Programmable; fully automated; low-cost; scalable for molecular diagnostics	Moderate fabrication complexity; not yet adapted for single-cell resolution	High	120, 121
OET & pOET	SU8 fabricated-microrobot	Transcriptome, cell–cell interaction	Optoelectronic tweezers	Cells, particles	Programmable; non-contact; minimal cell damage	Requires high-intensity light sources; limited speed and thermal effects	High	124
Optically actuated soft microrobot	TPP-fabricated microrobot	Cell manipulation, cell–cell interaction	Optical tweezers	Cells, particles	Low mechanical damage, high spatial precision, shape-adaptive	Complex fabrication; low actuation speed limits real-time applications	Low	125

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Table 2 Commercial microfluidics platforms for single-cell analysis, isolation and dispensing

Category	Platform	Brand	Principle	Automation level	Throughput	Applications	Features
Single-cell analysis	BOXmini SCP	ACX Instruments	Digital microfluidics	High	High (1536 cells per run)	Proteomics	High-throughput single-cell proteomics sample preparation
	Chromium	10× Genomics	Droplet microfluidics	High	High (hundreds to millions of cells per run)	Multi-omics	High-throughput droplet-based single-cell encapsulation; supports scrna-seq, scatac-seq, multiome
	Beacon	Bruker	Optofluidics	High	High (500 to 60 000 cells per run)	Genomics, transcriptomics	Optofluidic live-cell culture and functional profiling; for antibody discovery, vaccine and t-cell screening
Single-cell isolation and dispensing	CellShepherd	ARRALYZE	Microarray-based[200]	Medium	Low (1 cell per minute)	General cell applications	Imaging-based cell tracking and isolation; AI-driven analysis
	Cell Handler	Yamaha	Wellplate and capillary	Medium/high	Medium (8-channel tips; 96-/384-well plate)	General cell applications	Automated imaging and single-cell picking arm; Ai-integrated analysis, high resolution imaging
	CellenONE	SCIENION	Capillary droplet generation	High	High (96 cells isolation/3 minutes)	Multi-omics	High-accuracy image-based isolation; temperature control; omics-compatible
	F.SIGHT OMICS	Cytena	Cartridge microfluidics	Medium/high	High (384 cells isolation/8 minutes)	Multi-omics	Morphology- and fluorescence-based sorting; picoliter droplet generation
	DispenCell S4	SEED Biosciences	Wellplate and capillary	Low/medium	Medium (96 cells isolation/4 minutes)	General cell applications	Tip-based monoclonal cell isolation; semi-automated; for cell line development
	CellRaft AIR System	Cell Microsystem	Microraft array	Medium	Low (96 cells isolation/1.5 hours)	General cell applications	Raft array-based clone identification; enhanced cell outgrowth
	CellCelector	Sartorius	Wellplate and capillary	Medium/high	Low (1 cell isolation/20–30 seconds)	General cell applications	Image-guided robotic picking; monoclonal selection; well/nanowell compatibility

begun to emerge. In addition to DMF, Zhang *et al.* recently introduced an acoustofluidic liquid handling platform, PULSE, which enables precise, programmable ejection of nanodroplets and single cells *via* ultrasonic energy.[129] Collectively, robotic and commercial systems represent two complementary directions in single-cell microfluidics: customized robotic platforms offer flexibility and depth for specialized applications, whereas commercial robotic solutions provide robust, standardized workflows suitable for broader adoption in research and clinical settings. As integration between robotics, microfluidics, and downstream omics continues to evolve, these platforms are poised to play a central role in the next generation of single-cell analysis.

3. Deep learning for single-cell analysis

Deep learning has transformed single-cell analysis by enabling the extraction of meaningful insights from large-scale datasets. Convolutional neural networks (CNNs),[130] generative adversarial networks (GANs),[131] and variational autoencoders (VAEs)[132] have emerged as key frameworks in this transformation. CNNs (Fig. 5A), which excel in image-based tasks, capture spatial hierarchies of cellular features, making them indispensable for classification and regression models. Classification models categorize cells based on morphological and molecular characteristics, while regression models predict continuous variables such as gene expression levels or phenotypic responses. Generative models, including GANs (Fig. 5B) and VAEs, further expand analytical capabilities by generating synthetic single-cell data, correcting batch effects, and simulating cellular states under diverse conditions. By integrating these advanced deep learning approaches, single-cell analysis achieves unprecedented accuracy and scalability, driving new discoveries in cellular heterogeneity and disease progression. Building on the transformative applications of deep learning in single-cell analysis, we will review key advancements in classification, regression, generative modeling, and data integration, highlighting

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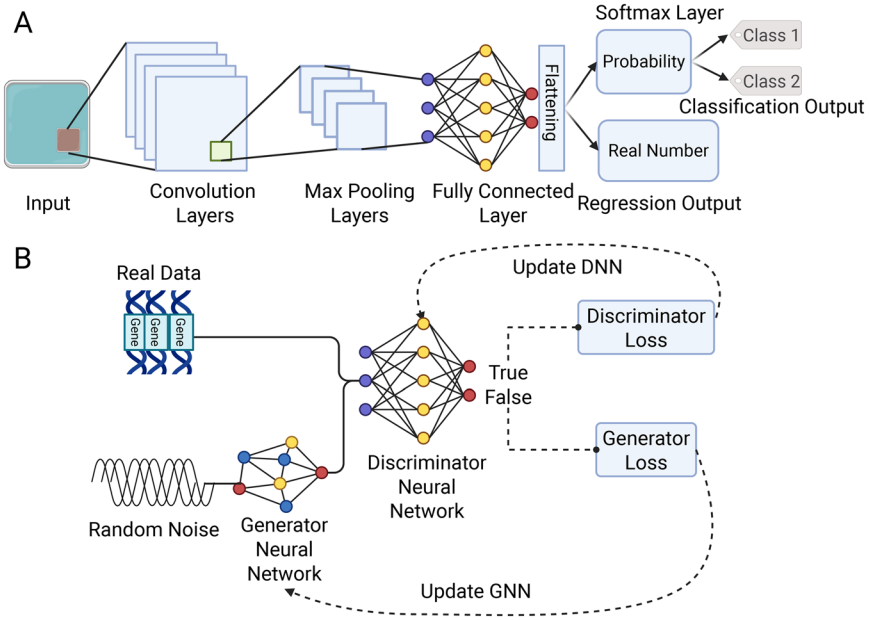

Fig. 5 AI models for single-cell analysis. (A) CNNs capture spatial hierarchies of cellular features, enabling precise cell classification and regression. Classification models distinguish cells by morphology and molecular traits, while regression models predict continuous variables like gene expression and phenotypic responses. (B) GANs consist of a generator that creates synthetic data and a discriminator that differentiates real from fake, engaging in an adversarial process to refine data generation. This framework enables high-fidelity single-cell data synthesis, correcting batch effects, and simulating cellular states under diverse conditions. Images were created using BioRender.

their impact on cellular characterization and predictive modeling (Table 3).

3.1. Classification of cell states and fates

Classification models are essential for distinguishing cell states and fates in single-cell analysis. CNNs have been particularly effective in the recognition of cellular morphology with high precision. Deep learning methods like Cellpose and LIVECell have advanced cell segmentation in microscopy images. Cellpose,[133,134] trained on over 70 000 segmented objects, enables precise segmentation across diverse image types without retraining. LIVECell,[135] a high-quality dataset of 1.6 million phase-contrast images, supports training models for accurate segmentation in variable cell morphologies and densities, improving high-throughput imaging. In addition, Din and Yu advanced single-cell segmentation by developing deep learning models capable of delineating cell boundaries with minimal human annotation.[136]

With segmented individual cells, there are multiple works that classify cell viability and damage severity, facilitating high-efficiency toxicity evaluation and the screening of potential anticancer drugs. For examples, Tox_(R)CNN was established by Jimenez-Carretero *et al.* to predict toxicity from images of DAPI-stained cells.[137] Chen *et al.* developed a deep learning model that predicts tumorsphere formation on day 14 based on day 4 images, significantly accelerating cancer stem-like cell identification.[138] Similarly, Pattarone *et al.* established a deep learning framework for classifying live and dead breast cancer cells without staining, expanding the applicability of label-free cell classification.[139] Ulicna *et al.* developed a Bayesian-based single-cell tracking approach that performs lineage tree analysis, by utilizing cell state classification which in turn improves tracking of cells in both 2D and 3D environments.[140] Hartnett *et al.* introduced live, apoptotic, and necrotic cell explorer (LANCE), a CNN-based model that classifies apoptotic and necrotic cells based on brightfield microscopy images, with an accuracy of 96.3 ± 0.5%, eliminating the need for fluorescence labeling.[141] He *et al.* developed a deep learning model, named detector of mitosis, apoptosis, interphase, necrosis, and senescence (D-MAINS), which leverages cellular morphology in phase contrast images for accurate cell status classification.[142] Li *et al.* developed an AI-based approach for detecting cell damage, utilizing a CNN trained on time-series fluorescence images collected before and after drug exposure, achieving an accuracy of over 93%.[143] These breakthroughs underscore the power of deep learning in advancing classification tasks for single-cell analysis. However, challenges persist in dataset annotation, model interpretability, and the generalizability of predictive accuracy across diverse cell models. To address these limitations, strategies such as transfer learning[144,145] and self-supervised learning[146,147] are being explored to enhance model robustness across diverse experimental conditions.

3.2. Regression to assess continuous biological variables

Regression models play a pivotal role in single-cell analysis by predicting continuous biological variables, offering deeper quantitative insights beyond discrete classification. These

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Table 3 Recent deep learning methods in single-cell analysis across segmentation, classification, regression, and generative modeling

Category	Source (year)	Models/methods	Sample type	Key advantage/findings	Applications	Limitations
Cell segmentation	Cellpose 2.0 (2021)[133]	DNN + U-Net style architecture + residual blocks	Fluorescence, and phase-contrast microscopy images	Provides a pretrained model zoo to accommodate various microscopy modalities; uses a human-in-the-loop approach to simplify model generation	Single-cell segmentation for diverse microscopy data	Human-in-loop approach may be less effective for complex morphologies
	LIVECell (2021)[135]	CNN-based segmentation model	Phase-contrast microscopy images	Enables label-free segmentation across diverse cell densities and morphologies	Single-cell segmentation and tracking in live-cell imaging	Performance depends on imaging quality; less effective for non-adherent cells
	Din *et al.* (2021)[136]	Self-supervised CNN segmentation model	Bright-field and fluorescence single-cell microscopy	Achieves accurate segmentation without manual labels or extensive supervision	Preprocessing for pipelines across modalities	Sensitive to cell density and imaging conditions; cannot distinguish cells from background in certain cases
Classification	Pattarone *et al.* (2021)[139]	CNN classifier (live *vs.* dead cells)	Bright-field breast cancer cell images	Achieves AUC of 0.94–0.98 without staining; suitable for live-cell analysis	Label-free viability screening	Specific to single cell lines; staining or capture methods may limit generalizability
	Ulicna *et al.* (2021)[140]	U-Net + classifier + Bayesian tracking	Time-lapse bright-field and fluorescence microscopy images	Combines cell classification with tracking to improve cell lineage analysis accuracy	Lineage tracking; single-cell studies	Requires high-quality time-lapse imaging
	Hartnett *et al.* (2022) – LANCE[141]	CNN classifier	Label-free bright-field images of cell lines	Provides 96.3 ± 0.5% accuracy in label-free bright-field imaging; tracks cell dynamics non-destructively	Cell death monitoring; cytotoxicity assessment	Sensitive to imaging conditions; may misclassify certain cell states
Regression	Green *et al.* (2021)[149]	DNN + cGAN	Zebrafish assay HTS chemical data	Achieves AUC of 0.837 in consensus models; improves sensitivity in chemical screening	Drug toxicity screening	Requires accurate 3D chemical structures; cannot evaluate mixtures
	Pham *et al.* (2021/22)[150,151]	GCN + feed forward neural network + interaction network + prediction network	LINCS L1000, STRING, DrugBank, patient expression SARS-CoV-2	Outperforms state-of-the-art methods; suitable for drug repurposing	Drug repurposing; prediction of gene expression changes	Strongly influenced by input data quality
	Chiang *et al.* (2024)[153]	CNN regression model	Phase-contrast images of microfluidic spheroids	Achieves correlation of r = 0.989 with LIVE/DEAD assays; transferable across laboratories	High-throughput drug screening	Imaging conditions may affect prediction
Generative modeling	Palma *et al.* (2025)[158]	Generative modeling for morphology prediction	Single-cell images under perturbations	Predicts morphological changes under drug or genetic perturbations	*In silico* screening of morphological effects	Requires diverse perturbation training datasets
	Xu *et al.* (2020) – scIGANs[160]	GAN-based imputation model	Single-cell RNA-seq data	Improves imputation accuracy and reduces sparsity in scRNA-seq data	Enhanced scRNA-seq analysis and clustering	Sensitive to input data quality; risk of over-smoothing
	Lopez *et al.* (2018)[163]	VAE	Single-cell RNA-seq data	Learns latent structures; supports clustering and trajectory inference	Unsupervised transcriptomics analysis	Interpretation challenges; computationally intensive
	Marouf *et al.* (2020)[201]	GAN/cscGAN	Single-cell RNA-seq data	Generates realistic scRNA-seq profiles; improves downstream analysis	Data augmentation; rare-cell analysis	Susceptible to mode collapse; high computational demand

methods are particularly valuable for high-throughput studies, where understanding heterogeneous cellular responses is essential. Zhang *et al.* applies random decision forest and artificial neural network to microfluidic single-cell migration data, and the model achieved high accuracy in predicting cell movement direction (over 99%) and speed (91%), identifying key morphological features that drive cell migration and metastasis.[148] Green *et al.* leveraged deep

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neural networks (DNNs) and conditional generative adversarial networks (cGANs) to predict toxicological responses from large-scale screening data, improving drug candidate prioritization, achieving an area under the receiver operating characteristic of 0.837.[149] Pham *et al.* introduced DeepCE, a deep-learning framework that predicts chemical-induced gene expression changes in a cell-type-specific manner, facilitating drug discovery and toxicology assessments and supporting drug repurposing for COVID-19 (ref. 150 and 151) Lakkis *et al.* introduced single cell imputation protein embedding neural network (sciPENN),[152] a versatile deep learning framework designed for the integration of CITE-seq and scRNA-seq data, enabling protein expression prediction for scRNA-seq and protein expression imputation for CITE-seq. Zhang *et al.* (1920 tumor spheres) and Chiang *et al.* (12 000 spheroids per chip) leveraged deep learning for label-free viability assessment of cancer spheroids in microfluidic platforms, reducing reliance on fluorescence staining while maintaining high classification accuracy.[153,154] Ma *et al.* systematically predicted the efficacy of >6000 compounds against therapy-resistant polyploid giant cancer cells.[155] Despite their promise, single-cell regression models face challenges such as biological noise, batch effects, and variability in experimental conditions. To mitigate these issues, the incorporation of more diverse single-cell datasets, along with the application of data augmentation, ensemble learning, and regularization techniques, is anticipated to improve model robustness and enhance predictive accuracy.

3.3. Generative modeling for single-cell analysis

Generative models, including GANs and VAEs, have emerged as powerful tools in single-cell analysis, enabling data augmentation, image denoising, and cellular behavior simulation. These models significantly enhance computational biology by facilitating synthetic data generation and improving analytical methodologies. In image-based single-cell analysis, Witmer and Bhanu employed GANs for dataset augmentation,[156] improving classification accuracy and achieving a 2% increase in both true positive rate and F1-score compared to imbalanced datasets. Similarly, Wu *et al.* developed a GAN-based method to generate synthetic images of human cardiomyocytes at various maturation stages,[157] enhancing classification accuracy and cellular structure analysis, outperforming conventional machine learning approaches. Palma *et al.* introduced the image perturbation autoencoder (IMPA)[158] to predict morphological changes in response to genetic and chemical perturbations, improving high-content screening analysis by accounting for batch effects and technical variations, making it a valuable tool for drug discovery. Additionally, Ternes *et al.* developed a multi-encoder VAE,[159] improving single-cell image analysis by extracting biologically relevant features, enhancing cell population separation, phenotypic distinctions, and correlation with other analytical methods. Beyond morphological analysis, generative models also aid in scRNA-seq, which, despite its ability to characterize transcriptomic profiles at high throughput, faces challenges such as dropout errors and technical noise. To address these limitations, GANs, including scRNA-seq imputation GANs (scIGANs)[160] and conditional single-cell GANs (cscGANs),[161] generate realistic imputed or augmented single-cell RNA-seq data, improving downstream analyses, rare cell population detection, and classification robustness. These models also mitigate data sparsity, enhance expression quality, and reduce reliance on biological samples, improving reproducibility and cost efficiency in biomedical research. Overall, GANs and VAEs are transforming single-cell analysis by improving data integrity, addressing technical limitations, and expanding analytical capabilities, paving the way for more accurate and scalable biomedical research.

3.4. The role of AI in enhancing single-cell data integration and analysis

AI is also playing an increasingly central role in the integration of multi-omics single-cell data by addressing challenges spanning data generation, integration, storage, analysis, and interpretation. In data generation, AI-driven experimental design and active learning methods help optimize sampling strategies, enabling researchers to prioritize informative cell populations or experimental conditions that maximize biological insight while reducing experimental costs and redundancy.[162] For example, adaptive sampling algorithms can guide iterative single-cell RNA sequencing to focus on rare or transitional cell states. In the realm of data integration, cutting-edge AI models such as variational autoencoders (*e.g.*, scVI[163] and TotalVI[164]) and graph neural networks[165,166] have proven effective at aligning and integrating diverse modalities including transcriptomics, proteomics, and epigenomics, enabling comprehensive characterization of cellular states across multiple molecular layers. These models can overcome batch effects and technical noise, harmonizing datasets collected across different platforms or time points.

Scalable data storage, transfer, and archiving infrastructures, combined with standardized metadata frameworks (such as the Human Cell Atlas metadata standards[167,168]), are critical to support reproducible AI-driven analyses and facilitate large-scale data sharing. To enable efficient AI applications, curated and well-annotated databases, like the Single Cell Expression Atlas[169] and Tabula Muris,[170] are developed with AI-friendly formats, accompanied by modular, reproducible pipelines (*e.g.*, Scanpy[171] and Seurat[172]) that streamline preprocessing and downstream machine learning analyses. AI-powered quality control tools automatically detect technical artifacts, low-quality cells, and batch effects; methods like DoubletFinder[173] and SoupX[174] are widely used to clean single-cell datasets prior to analysis.

Importantly, advances in explainable AI are making it possible to interpret complex models and extract biologically

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meaningful insights. For example, CellOracle[175] uses machine learning to infer gene regulatory networks and predict cellular responses to perturbations, providing mechanistic insights rather than black-box predictions. Similarly, scMoMaT[176] integrates multi-omics single-cell data to identify coordinated regulatory programs, facilitating hypothesis generation grounded in interpretable features. Together, these developments demonstrate AI's transformative potential to not only enhance technical aspects of data handling but also to enable deeper biological discovery through integrative and interpretable analyses in single-cell and multi-omics research.

3.5. Barriers to reliable AI in single-cell microfluidics

Despite their impressive performance, current AI pipelines for single-cell imaging and microfluidic read-outs still suffer from a number of limitations that negatively affect scientific transparency and reproducibility.[177] The deeply layered, "black-box" nature of CNNs, GANs, and transformer models obscures the mechanistic links between an input image and the model's prediction, making it hard for researchers or regulators to verify AI-generated hypotheses or understand the thought process behind certain classifications or features that might be selected for training the models. Because models typically demand large, expertly annotated datasets, any bias or noise introduced during annotation, image acquisition, or batch processing can propagate through training and destabilize downstream results. Even when sizeable datasets exist (*e.g.*, Cellpose or LIVECell), differences in microscope settings, cell lines, or microfluidic chip geometries often erode model generalizability, forcing each laboratory to perform costly re-training and hyper-parameter tuning that are rarely described in sufficient detail for others to reproduce. The heavy computational footprint of state-of-the-art networks further complicates transparency: model weights, training logs, and version-specific software dependencies are rarely saved, so a result obtained on one graphics processing unit (GPU) stack may be irrecoverable on another. Finally, the field lacks standardized benchmarks for evaluating continuous-variable regressors (*e.g.*, viability scores or migration velocities), leaving researchers to report disparate metrics that impede cross-study comparison. Addressing these gaps will require open, modality-matched reference datasets, shareable training pipelines with locked random seeds and environment files, and the wider adoption of interpretable, self-supervised, or transfer-learning frameworks that can be audited and stress-tested across laboratories before their biological conclusions are trusted.

4. Potential role of cloud lab concepts in single-cell experimentation

Despite the transformative potential of integrating microfluidics, robotics, and AI in single-cell analysis, technical and operational challenges remain significant. These complexities have prompted interest in a new envisioned model: the cloud lab.[178–180] Cloud labs (Fig. 6) have been described as centralized facilities equipped with sophisticated instrumentation and infrastructure that researchers can access remotely.[181–183] Through cloud interfaces, scientists can submit standardized protocols and samples, receive real-time or asynchronous experimental outputs, and conduct downstream computational analyses,

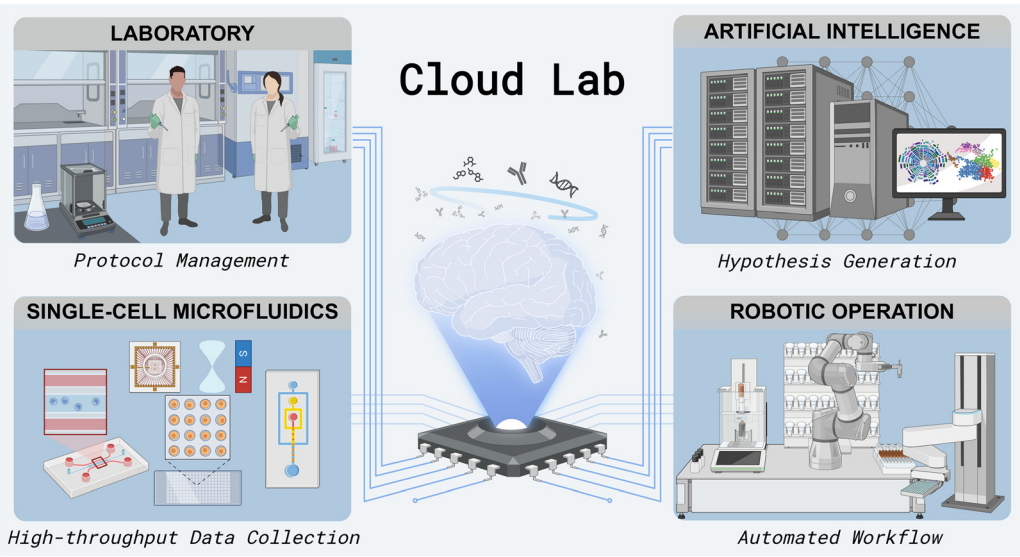

Fig. 6 Cloud Lab synergizing microfluidics, robotics, and AI for accelerated single-cell discovery. The Cloud Lab concept connects laboratory protocol management, single-cell microfluidics, robotic operation, and artificial intelligence into a unified workflow. Laboratory modules manage experimental protocols, while microfluidic systems enable high-throughput single-cell data collection. Robotic platforms execute automated workflows, and AI modules generate and refine hypotheses based on acquired data. Images were created using BioRender.

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enabling a degree of experimentation without direct physical presence. This model holds promise for supporting single-cell workflows, where robotic execution of highly repetitive and sensitive steps, such as droplet generation, reagent dispensing, and high-content imaging, could benefit from automation and central standardization if realized. In practice, however, many upstream elements of single-cell experiments, such as microfluidic chip fabrication, reagent formulation, and biological sample preparation, still demand expert manual handling and cannot yet be fully automated. As such, cloud labs should currently be seen as complementary to traditional laboratories, providing infrastructure for partial automation, protocol standardization, and collaborative data analysis, rather than serving as complete replacements. Cloud labs offer advantages in enhancing reproducibility and scalability in robotic-driven workflows. By centralizing the execution of experimental protocols and integrating with cloud-based data processing pipelines, they can minimize human variability in routine tasks and improve consistency across different users and institutions. For example, robotic liquid handling and imaging systems operated within cloud lab environments can deliver consistent performance under tightly controlled conditions, reducing experimental drift over time and variations between laboratories, which is critical for deep learning data analysis.

From a collaborative perspective, cloud labs are especially useful within structured partnerships or consortia, where institutions can share expertise and infrastructure under clearly defined governance models. In such frameworks, cloud labs act as intermediaries, allowing remote users to leverage specialized instrumentation managed by trained personnel. Commercial platforms (*e.g.*, Strateos, Emerald Cloud Lab) have provided early demonstrations to operate this model through subscription or usage-based services, offering access to robotics and analytical infrastructure for research groups without direct access to such resources. While the financial expense of cloud lab access varies depending on experimental complexity and support needs, this shared-access model has the potential to lower entry barriers for technically demanding workflows such as single-cell assays.

Importantly, while cloud labs offer a forward-looking infrastructure, claims that they can fully eliminate physical laboratory needs or democratize all aspects of experimental biology should be considered in light of real-world constraints, including cost, technical complexity, and the need for real-time decision-making in dynamic biological experiments. A more realistic and achievable path lies in hybrid integration, in which cloud-connected systems enhance reproducibility, enable remote collaboration, and operate standardized components of experiments, while still relying on local expertise for complex, hands-on steps. Overall, cloud labs represent an envisioned framework that may, in the future, support single-cell research, particularly in the realms of standardized data acquisition, robotics, and inter-institutional collaboration. While challenges remain in areas such as device fabrication and biological sample handling, cloud labs may ultimately augment traditional laboratory workflows, acting as powerful facilitators of experimental rigor, scalability, and accessibility.

5. Conclusion and perspective: challenges and opportunities in integrating AI and robotics for advancing single-cell analysis

Currently, AI and robotics are transforming single-cell research, with their potential realized through integration across three critical domains: (1) robotic generation of large-scale datasets for deep learning, (2) rapid validation of novel hypotheses generated by AI, and (3) iterative feedback to enhance model refinement. Robotics is indispensable for creating high-quality, large-scale datasets required to train and optimize AI models. For example, microfluidic platforms can process thousands of single cells simultaneously, generating vast datasets for transcriptomics, proteomics, or metabolomics. These datasets feed into AI algorithms, sharpening their predictive capabilities and enabling the generation of increasingly complex and innovative hypotheses. Recent developments in deep visual proteomics (DVP)[184] and single-cell DVP (scDVP)[185] demonstrate how AI-guided image processing and robotic microdissection can link spatial cellular phenotypes to proteomic profiles at single-cell resolution, uncovering spatially regulated disease mechanisms in complex tissues. These approaches illustrate the promise of this technological convergence.

However, this vision is also constrained by both technical and conceptual limitations. One major challenge is the annotation burden: biological datasets often require expert labeling to train supervised models, which become particularly onerous in single-cell contexts where morphological, molecular, and functional heterogeneity is high. Moreover, many AI models, especially deep neural networks, function as "black boxes", raising concerns about interpretability and the transparency of biological insights they generate. Without a clear understanding of how predictions are derived, it can be difficult to assess their scientific validity or clinical relevance. Furthermore, even with automation, reproducibility in AI-driven workflows remains a concern, particularly when predictions rely on complex models trained with non-standardized preprocessing pipelines or limited sample diversity. These issues may undermine confidence in AI-generated hypotheses unless carefully addressed through rigorous experimental design and benchmarking.

Looking ahead, the iterative feedback loop between AI and robotics represents an envisioned paradigm for biomedical research (Fig. 6). Robotic experimentation generates data that improves AI models, while AI refines hypotheses that drive further experimentation. This self-reinforcing cycle, although

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not yet fully realized, could accelerate discovery, enabling deeper insights and breakthroughs that would be unattainable with either technology alone. Together, AI and robotics are poised to revolutionize single-cell analysis and redefine biomedical innovation. The symbiotic relationship between AI and robotics can also extend to the development of new experimental techniques. Robotics can facilitate the design and testing of novel methodologies proposed by unsupervised AI models.[186] For instance, AI might suggest unconventional ways to sort or label cells based on subtle, previously unrecognized features through unsupervised learning. Robotic platforms can implement these techniques, generating data that not only validates the methodology but also expands the boundaries of what is experimentally possible. This iterative process continuously pushes the limits of single-cell analysis, enabling breakthroughs that were previously inconceivable.

Ultimately, the convergence of microfluidics, robotics, and AI is poised to reshape single-cell analysis. While each of these technologies has independently contributed to advances in throughput, precision, and discovery, their true transformative power lies in their integration. However, the field remains fragmented, with relatively few platforms offering end-to-end solutions from sample preparation to hypothesis generation and validation. We anticipate a shift from Siloed tool development to modular, interoperable ecosystems, where standardization, cloud-based experimentation, and real-time AI feedback loops will be central. Importantly, the next phase of innovation will demand more than technical optimization; it will require frameworks for interpretability, reproducibility, and deployment of autonomous decision-making in biological research. Rather than a linear progression, we foresee the field evolving into a multidimensional space, one that combines biological insight, engineering, and responsible AI. In this light, integration should be viewed not as an accomplished reality, but as a forward-looking vision that redefines how discovery in life sciences may be achieved in the future.

Abbreviations

AM-DMF-SCP	Active-matrix digital microfluidic chip for single-cell proteomics
AI	Artificial intelligence
AIBO	Artificial intelligence robot
ALPS	Automated live imaging and cell picking system
BERT	Bidirectional encoder representations from transformers
CITE-seq	Cellular indexing of transcriptomes and epitopes by sequencing
CCD	Charge-coupled device
cscGANs	Conditional single-cell GANs
cGAN	Conditional GAN
CNN	Convolutional neural network
CVs	Coefficients of variation
DIA	Data-independent acquisition
DNN	Deep neural network
DVP	Deep visual proteomics
DMF	Digital microfluidics
DISCO	Digital microfluidic isolation of single cells for -omics
DEP	Dielectrophoresis
DEP-dTNT	Dielectrophoresis-trapping-nanowell-transfer
DLP	Direct library preparation
DASEI-MS	Droplet-assisted electrospray ionization-MS
Drop-seq	Droplet-based single-cell RNA Sequencing
EWOD	Electrowetting-on-dielectric
FACS	Fluorescence-activated cell sorting
GAN	Generative adversarial network
GPU	Graphics processing unit
IMPA	Image perturbation autoencoder
LoC	Lab-on-a-chip
LLM	Large language model
LC–MS	Liquid chromatography–mass spectrometer
LANCE	Live, apoptotic, and necrotic cell explorer
MACS	Magnetic-activated cell sorting
MS	Mass spectrometry
MALDI MS	Matrix-assisted laser desorption/ionization-MS
nanoPOTS	Nanodroplet processing in one pot for trace samples
OT	Optical tweezer
OET	Optoelectronic tweezer
p-OET	Patterned OET
PR2	Personal robot 2
PiSPA	Pick-up single-cell proteomic analysis
POCT	Point-of-care testing
PDMS	Polydimethylsiloxane
RAEE	Robot-assisted acoustofluidic end effector
RNA	Ribonucleic acid
RAD microfluidics	Robotics automation of droplet microfluidics
ROS	Robot operating system
RT-1	Robotic transformer-1
SIPP	Safe interval path planning
scIGANs	scRNA-seq imputation GANs
scRNA-seq	Single-cell RNA sequencing
SciProChip	Single-cell integrated proteomic microfluidic chip
sciPENN	Single cell imputation protein embedding neural network
SODA	sequential operation droplet array
scDVP	Single-cell DVP
TMT	Tandem mass tag
tDISCO	Tissue-DISCO
TILs	Tumor-infiltrating lymphocytes

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TPP	Two-photon polymerization
VAE	Variational autoencoder
VI	Variational inference
YOLO	You only look once

Author contributions

Jinxiong Cheng: conceptualization, visualization, writing – original draft, writing – review & editing. Rajiv Anne: conceptualization, visualization, writing – original draft. Yu-Chih Chen: conceptualization, funding acquisition, project administration, resources, supervision, writing – original draft, writing – review & editing.

Conflicts of interest

The authors declare that no competing interests exist.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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