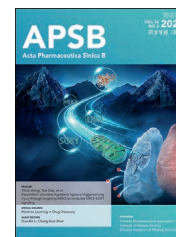




Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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REVIEW

AI-integrated microfluidics for drug screening: From single cell to organ-on-a-chip



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Received 11 July 2025; received in revised form 6 September 2025; accepted 15 October 2025

KEY WORDS

Microfluidics;
Artificial intelligence;
Drug screening;
Organ-on-a-chip;
Machine learning

Abstract Drug discovery remains a protracted and capital-intensive process, primarily hindered by inefficiencies in drug screening. Microfluidic technology provides a promising approach for *in vitro* drug screening, enabling physiologically relevant, high-throughput, and cost-effective analysis by mimicking key aspects of cellular microenvironments. The synergistic integration of artificial intelligence (AI) with microfluidics constitutes a pivotal advancement in biomedical analysis. The convergence of the two facilitates automated data analysis, complex pattern recognition, and intelligent experimental control, thereby accelerating drug screening and contributing to enhanced precision. This review systematically presents the latest advancements in AI-assisted microfluidic drug screening, organized by increasing biological complexity: from single-cell analysis (1D), multicellular arrays (2D), and spheroids (3D), to sophisticated Organ-on-a-chip (OoC, 3D+) platforms. We detail how AI algorithms promote screening throughput, sensitivity, and physiological relevance at each scale. Furthermore, we critically discuss the prevailing challenges, including those related to data, model robustness, interpretability, and system integration. Finally, we outline future directions, highlighting the potential of AI-enhanced microfluidics to further advance precision drug discovery and biomedical research. We believe this timely review will offer a useful reference for researchers working in the interdisciplinary field of AI, microfluidics, and pharmacology.

This article is part of special issue entitled: Machine Learning in Drug Discovery published in Acta Pharmaceutica Sinica B.

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Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

<https://doi.org/10.1016/j.apsb.2026.01.001>

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1. Introduction

Despite continuous innovation, drug discovery remains a notoriously lengthy, costly, and unpredictable process^{1–3}. A major issue causing this problem is the inefficiency of drug screening, which leads to high attrition rates: only around 12% of drug candidates entering clinical trials ultimately receive regulatory approval, with market launch rates often below 10% and average development costs exceeding 2.6 billion USD per drug^{4–6}. These alarming statistics highlight the significant challenge of effectively translating early-stage findings into clinical validation^{7–9}, emphasizing the urgent need for more efficient and reliable screening approaches. High-throughput screening (HTS) has emerged as an indispensable tool for accelerating this process by enabling rapid evaluation of compound efficacy and safety^{10–12}. However, conventional HTS methods, including traditional cell-based assays and animal models, suffer from significant limitations such as inadequate physiological fidelity, challenges in high-throughput scaling, and compromised predictive accuracy^{13,14}. These shortcomings, coupled with growing ethical concerns regarding animal use, highlight the imperative for more accurate, efficient, and ethically sound screening platforms.

Against this backdrop, microfluidics-based technologies have emerged as a promising approach, providing notable advantages over traditional drug screening modalities^{15–17}. By precisely manipulating fluid volumes from picoliters to milliliters within microscale environments, microfluidics provides key benefits for drug screening. These advantages include minimal reagent consumption, high throughput, and the ability to mimic native cellular microenvironments^{18–20}. Furthermore, these systems enable dynamic drug delivery, real-time monitoring of cellular responses, and parallel screening of compound libraries across dose gradients and combinations. Consequently, microfluidic platforms have been widely adopted for drug screening, accelerating hit identification and lead optimization while improving physiological relevance²¹. However, the complex and high-dimensional datasets generated in these microfluidics-based experiments, which encompass cellular behaviors, drug responses, and spatiotemporal gradients, present significant challenges for efficient analysis and

interpretation²². Extracting actionable insights from such intricate data is crucial for informing drug screening decisions and optimizing pharmaceutical development, thereby necessitating robust computational frameworks capable of processing heterogeneous and multimodal inputs²³.

Artificial intelligence (AI), particularly machine learning (ML)²⁴ and deep learning (DL)²⁵, offers a valuable approach to current bottlenecks in drug discovery¹⁸. Leveraging advances in computational power and algorithmic design, AI technologies are increasingly integrated across the entire drug discovery pipeline²⁶, serving as an effective component in data-intensive frameworks^{27,28}. This integration spans from target identification and efficacy prediction to toxicity assessment and biomarker discovery^{29–33}. In recent years, the convergence of AI with microfluidic platforms has profoundly reshaped conventional drug screening workflows, elevating throughput, precision, and automation^{16,18,23}. Within microfluidics-based screening, AI facilitates automated feature extraction, pattern recognition, and intelligent workflow control^{34,35}, which enables more accurate modeling of drug–cell interactions within physiologically relevant settings^{36–39}. Table 1 provides an overview of widely used AI methodologies in microfluidics-based drug screening, listing each method's full name, core function, and representative microfluidic applications. This summary serves as a conceptual reference point for readers, supporting later sections where these AI tools are discussed in the context of specific dimensional platforms and use cases. Concurrently, microfluidic platforms provide structured and highly controllable data environments that support the development and refinement of AI algorithms^{40,41}, fostering a mutually reinforcing system between hardware and computation. This synergistic integration of AI and microfluidics thus holds substantial promise for advancing drug screening by optimizing throughput, precision, and cost efficiency. Such developments are important for progressing towards personalized and stratified medicine⁴². Recently, AI-assisted microfluidic systems have demonstrated significant advances across various biological scales, from single cells (1D) and multicellular models (2D) to spheroids (3D), and more complex structures like organoids and OoC systems. These platforms not only improve the physiological relevance of *in vitro* models but also

Table 1 Overview of widely used AI methodologies in microfluidics-based drug screening.

AI method	Full name	Basic function	Typical application in microfluidics
CNN	Convolutional neural network	Automatically extracts spatial features from image or video data	Phenotypic image classification, cell morphology analysis
RNN	Recurrent neural network	Captures temporal dependencies in sequential data	Time-series drug response profiling, signal trajectory analysis
MLP	Multilayer perceptron	Fully connected neural network for general nonlinear mapping	Drug efficacy prediction from tabular data
SVM	Support vector machine	Finds optimal hyperplane to separate data classes	Binary drug classification (toxic vs. non-toxic)
PCA	Principal component analysis	Dimensionality reduction and data visualization	Feature compression, clustering of cellular responses
GNN	Graph neural network	Learns patterns from graph-structured data	Modeling cell–cell interactions or compound–target networks

contribute to accelerating the transition from *in vitro* screening to *in vivo* validation, indicating a shift in drug discovery from empirical to intelligent decision-making. To illustrate how AI tools are deployed across biological scales, from single-cell to OoC systems, Fig. 1 provides a schematic framework linking representative microfluidic models (1D–OoC) with relevant AI tasks and data types. This conceptual map helps contextualize later

discussions and highlights how AI adapts to different spatial complexities and data modalities.

This review provides a comprehensive overview of recent advancements in AI-assisted microfluidic platforms for drug screening. We systematically organize the content by increasing biological complexity. For each complexity level, we detail how AI integration enhances screening throughput, sensitivity, and

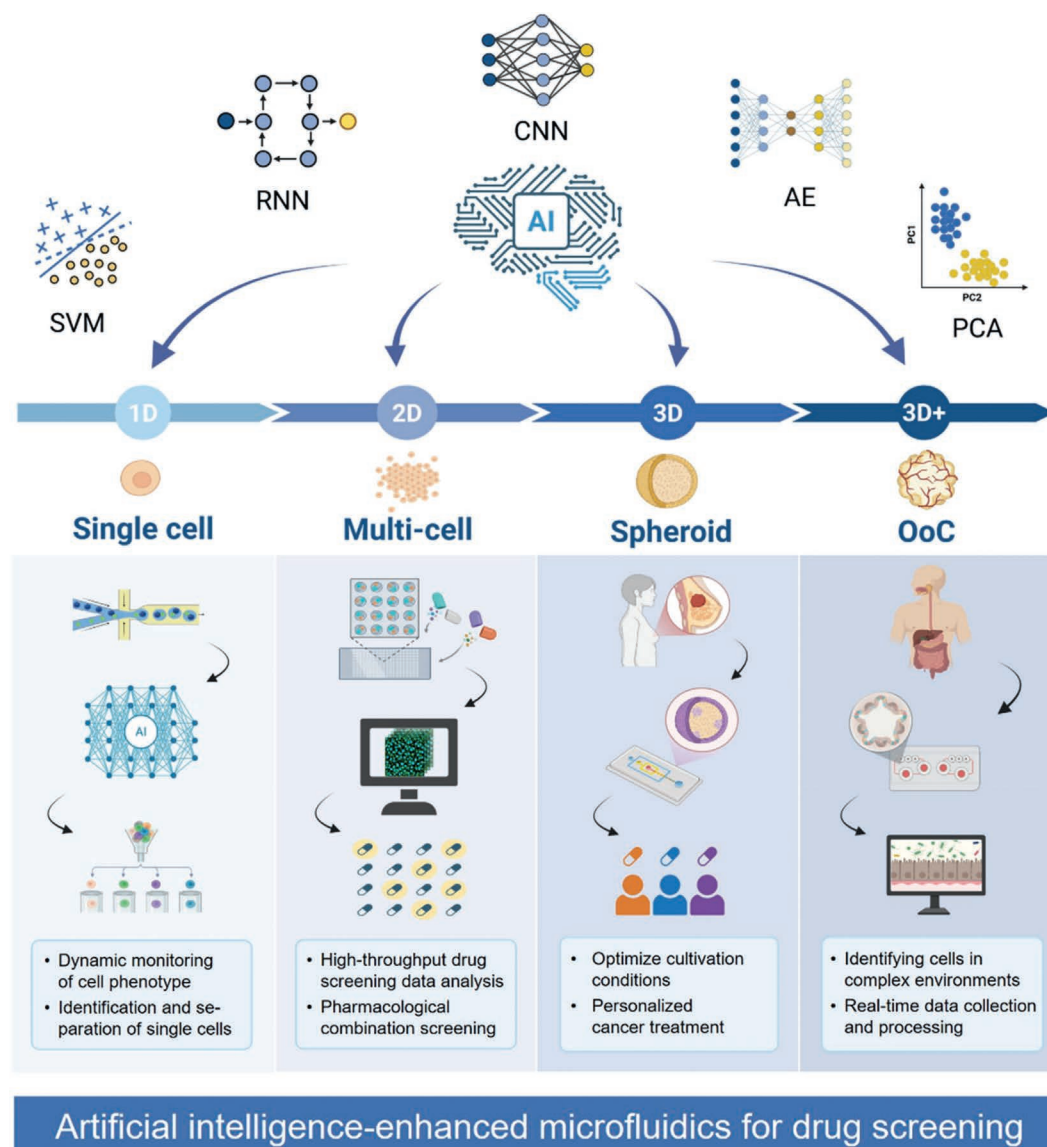


Figure 1 Schematic overview of AI-enhanced microfluidics for drug screening across increasing biological dimensions. 1D (single-cell analysis): Focuses on individual cellular responses, enabling the interrogation of cellular heterogeneity and rare cell populations. At this level, AI models such as convolutional neural networks (CNNs), recurrent neural networks (RNNs), and support vector machines (SVMs) are commonly applied to imaging and electrical signal data for cell classification, phenotype profiling, and real-time sorting. 2D (multicellular array): Extends to planar co-cultures and cell populations, allowing for the study of cell–cell interactions and collective behaviors. Here, CNNs and traditional machine learning methods are used to analyze morphology, migration patterns, and drug responses using multiparametric datasets. 3D (spheroid): Represents three-dimensional aggregates with tissue-like properties and microenvironments. AI facilitates viability estimation, morphological segmentation, and quality control through models such as Mask R-CNN applied to bright-field or phase-contrast images. OoC system (3D+): Recapitulates organ-level physiology by incorporating flow, mechanical forces, and complex multicellular structures. This level often involves complex, multimodal datasets (*e.g.*, high-content imaging, multi-omics, biosensing signals), requiring advanced AI architectures including graph neural networks (GNNs) and autoencoders for dynamic response prediction and intelligent system control. This Fig. is created with BioRender.com.

physiological relevance. Furthermore, we critically discuss the prevailing challenges in this field, such as those related to data, model robustness, interpretability, and system integration. Finally, we outline future directions and emerging trends for AI-enhanced microfluidics, aiming to identify areas for continued development that could further advance precision drug discovery and biomedical research.

2. AI-accelerated microfluidic drug screening at single-cell level (1D)

2.1. Microfluidics-based single-cell drug screening

Conventional drug screening often relies on population-level measurements using culture dishes or multi-well plates, which capture the average response of a cell population. While simple and scalable, such bulk analyses tend to obscure cellular heterogeneity, particularly rare subpopulations that are either highly sensitive or resistant to a given drug. As a result, critical biological signals may be masked, which compromises both mechanistic understanding and the accuracy of efficacy assessments. Cellular heterogeneity refers to the differences among individual cells within a seemingly homogeneous population⁴³. These variations may involve gene expression, molecular composition, morphology, and functional state. They can arise from diverse sources, including genetic background, epigenetic modifications, local microenvironment, or cell cycle stage⁴⁴. Such heterogeneity is widely observed even in clonal or morphologically uniform cell lines. Accumulating evidence shows that these differences play a central role in key biological processes such as tumor progression, stem cell differentiation, immune modulation, and drug resistance⁴⁵. In cancer, for instance, distinct subpopulations often exhibit divergent drug responses, which can ultimately influence therapeutic outcomes⁴⁶. Single-cell drug screening has emerged as a powerful approach for uncovering mechanisms of heterogeneity and identifying critical subpopulations^{47,48}. Unlike bulk assays, single-cell analysis allows direct observation of differential drug responses at the individual cell level, enhancing predictive accuracy and enabling early detection of resistant cells. This could help reduce treatment failure. However, the implementation of high-throughput and reproducible single-cell screening remains technically demanding⁴⁹. Key challenges include the efficient isolation and precise positioning of single cells, generation of stable drug gradients at the microscale, integration of multiparametric readouts, and the ability to analyze large, complex datasets.

Microfluidic technology offers an integrated platform to address these challenges. Compared with traditional methods, microfluidics supports high-throughput processing with minimal sample input, automated operation, and reduced risk of contamination⁵⁰. Its ability to manipulate single cells and finely tune their microenvironment is particularly advantageous in single-cell applications⁵¹. For example, droplet-based microfluidics can rapidly generate thousands of isolated microreactors, each encapsulating a single cell, thereby enabling parallel functional assessment at high resolution⁵². This approach has been used to quantify protease activity in tumor cells, improving both screening efficiency and data quality. To facilitate single-cell array construction, Li et al.⁵³ developed an open hydrogel-based microfluidic platform that enables high-density trapping of single cells, long-term culture, and *in situ* analysis. This system supports downstream applications such as genotyping and endpoint assays. Li's group⁵⁴ further integrated a concentration gradient generator into the array system,

allowing HTS of cancer drugs with enhanced efficiency and resolution. Beyond oncology, single-cell microfluidic platforms are increasingly applied in antibiotic resistance profiling^{55–58}, characterization of tumor heterogeneity^{59–62}, and studies of dynamic cellular responses⁵⁴. Collectively, these advancements highlight the increasing precision, scalability, and informational depth of single-cell drug screening. They reflect the growing maturity and versatility of microfluidic platforms as a critical bridge between basic research and translational applications, and lay a robust foundation for the integration of AI into high-resolution, single-cell-level drug discovery workflows.

Moreover, efficient identification and isolation of cells that develop drug resistance or exhibit atypical responses is essential for elucidating resistance evolution, tracking drug sensitivity dynamics, and uncovering therapeutic mechanisms. Compared with conventional approaches, microfluidic platforms offer distinct advantages for single-cell isolation^{63,64}, particularly in processing large sample volumes with high precision and automation, minimizing operator-induced variability. Recent advances, most notably droplet-based HTS systems⁶⁵ and chamber-based single-cell manipulation technologies⁶⁶, have significantly enhanced the efficiency and accuracy of rare-cell capture. Fiedler et al.⁶⁷ integrated droplet-based microfluidics with the instantaneous response of fluorescence sensors to achieve physical separation of target cells *via* dielectrophoresis. Similarly, Ung et al.⁶⁸ utilized surface acoustic wave (SAW)-based actuation to enable high-throughput, non-contact single-cell sorting. These capabilities are especially valuable for profiling functional heterogeneity at the single-cell level following drug exposure. By enabling the selective enrichment of small populations of resistant or uniquely responsive cells, such microfluidic strategies offer a practical and powerful means to identify emerging drug-resistant clones and to assess heterogeneous drug responses, making them indispensable tools for single-cell drug sensitivity evaluation.

2.2. AI methodologies for single-cell data analysis and manipulation

The application of microfluidic technology in single-cell analysis often generates massive amounts of data, encompassing rich biological information extracted from individual cells. These data come in complex and diverse forms, ranging from optical and electrical signals to microscopic images and time-series recording of dynamic cellular processes. Effectively unifying and accurately quantifying such heterogeneous data has become a significant analytical challenge. The integration of AI enables not only the efficient quantification and processing of complex data, but also improves analytical accuracy through multimodal data fusion, precise recognition, and robust classification⁶⁹. These capabilities significantly accelerate the analysis process, promoting the automation and standardization of microfluidic single-cell data analysis. Recent works have showcased the versatility of AI across imaging modalities, including fluorescence, bright-field, and holography, as well as signal processing domains, such as impedance and electro taxis. Accordingly, this section will elaborate on how AI methods are specifically employed to address these challenges, primarily focusing on advanced single-cell data analysis and enhanced cell isolation and sorting.

AI demonstrates significant advantages in processing single-cell microfluidic data, particularly in complex image and electrical signal analysis. The selection of an appropriate AI model is critical and often depends on the specific data characteristics, the

complexity of the analytical task, and crucially, the requirements for model interpretability. For instance, while DL models such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs) excel at automated feature extraction and processing large volumes of high-dimensional, unstructured data (e.g., high-resolution images and time-series signals), their inherent “black-box” nature often limits the direct interpretation of complex cellular responses, which is crucial for mechanistic understanding in drug development^{70,71}. Furthermore, training these sophisticated models for high-throughput single-cell applications can be computationally intensive, requiring substantial hardware resources. The limited availability of large, high-quality labeled single-cell datasets can also lead to issues like overfitting, hindering the models’ generalizability across different experimental setups or cell types. In contrast, traditional ML algorithms like support vector machines (SVM) or Random Forests can be more advantageous for smaller, structured datasets or when high model transparency is essential for understanding underlying drug mechanisms. These traditional ML models often offer greater interpretability, providing clearer insights into how specific biological parameters influence drug responses.

For image data analysis, AI is increasingly applied across various imaging modalities, including fluorescence, bright-field, and holography. It can handle complex image features such as overlapping spectral signals, intensity variations, and background noise, which are challenging for traditional analytical methods. Specifically, in high-throughput single-cell imaging, a prevalent challenge is maintaining a high signal-to-noise ratio (SNR) due to fast acquisition speeds and inherent biological variability. To mitigate this, AI-driven solutions are crucial for extracting reliable information from noisy data and ensuring analysis fidelity. For instance, self-supervised DL methods like SN2N enable effective denoising of live-cell super-resolution microscopy even from single noisy frames, significantly improving photon efficiency and mitigating artifacts⁷². Similarly, unsupervised adaptive DL frameworks enhance SNR and suppress background noise in light scattering imaging videos by leveraging temporal information, thereby improving single-cell classification and analysis⁷³. AI enables high-throughput, high-resolution single-cell drug screening by integrating cross-channel information, resolving signal interference, and extracting subtle phenotypic patterns⁷⁴. For instance, region-based CNNs like Mask R-CNN (Masked Region-based CNN) facilitate precise segmentation of cell images and extraction of key features⁷⁵, while YOLO (You Only Look Once) series models excel at real-time, accurate cell recognition and segmentation⁷⁶. Similarly, for electrical signal data analysis, AI is employed in the analysis of single-cell electrical signal data to achieve rapid and accurate assessment of drug efficacy. For example, ML algorithms can be used to quantitatively extract features like cell migration directionality, electrotactic alignment, and velocity, revealing the mechanisms of cellular response to electric fields⁷⁷. CNNs and RNNs can directly extract intrinsic dielectric properties from raw impedance data streams, resolving single-cell signals hidden within overlapping measurements and enabling real-time processing synchronized with fluid dynamics⁷⁸.

These sophisticated analytical capabilities also profoundly enhance single-cell manipulation and sorting. While traditional single-cell sorting methods (e.g., di-electrophoresis or SAW-driven) are effective, they often rely on relatively simple and predefined classification mechanisms, which limit their ability to accommodate complex cellular phenotypes. Furthermore, key parameters such as sorting thresholds often require manual

calibration or must be predetermined prior to experimentation, restricting the system’s adaptability to sample heterogeneity. By leveraging AI, researchers can now perform rapid, high-dimensional analysis of both morphological and molecular features across large cell populations at the single-cell level. More importantly, AI-driven models can autonomously optimize sorting thresholds and decision-making strategies based on training data, thereby enhancing system flexibility and enabling accurate, efficient isolation and manipulation of rare or phenotypically distinct single cells from heterogeneous samples. This is typically achieved by combining AI models with high-speed image acquisition systems and real-time mechanical actuators for image-guided cell sorting.

2.3. Applications of AI-assisted single-cell drug screening

Those refined AI methodologies introduced above, spanning both advanced data analysis and intelligent cell manipulation, are now broadly applied across single-cell drug screening. The following section will detail key applications, which are primarily categorized into two areas: AI for efficient single-cell phenotypic analysis and AI for enhanced single-cell sorting. Fig. 2 presents representative examples of these AI-driven systems, highlighting their diverse approaches to single-cell phenotypic analysis and enhanced cell sorting. And a summary of representative AI-integrated single-cell microfluidic platforms is provided in Table 2.

2.3.1. AI for efficient single-cell phenotypic analysis

High-precision tumor cell classification and morphological insights. Pirone et al.⁸¹ developed a ML-driven tomographic phase imaging flow cytometry system. This platform leverages microfluidic channels to induce controlled cell flow and rotation, enabling the acquisition of multi-angle phase images of single cells using digital holography. These images are then reconstructed into 3D refractive index maps, offering detailed morphological insights. A hierarchical classification model, consisting of two shallow neural networks, is employed to first distinguish tumor cells from non-tumor cells, and subsequently classify them as either neuroblastoma or ovarian cancer. This approach achieved an overall classification accuracy of 95.2%, with tumor cell identification and subtype classification accuracy both exceeding 97%, demonstrating strong potential for high-precision drug screening applications.

Identification of rare drug-resistant clones and evaluation of AML drug responses. Yellen et al.⁷⁵ developed a high-throughput single-cell phenotyping platform capable of profiling nearly 100,000 clonal cells per experiment through fluorescence imaging (Fig. 2A). By leveraging computer vision and ML techniques, specifically Mask R-CNN, the system enabled precise segmentation of cell images and extraction of key features such as cell count, morphology, and division rates. This approach allowed for the identification of rare ($\sim 0.05\%$) drug-resistant clones, enabling quantitative assessment of acute myeloid leukemia (AML) cell responses to targeted therapies. The platform successfully uncovered rare resistance phenotypes and distinct morphological patterns, offering a powerful tool for studying AML drug resistance and holding promise for broader applications across other cell models and disease systems.

Immunophenotyping. Kuo et al.⁷⁶ developed an integrated fluorescence-based microfluidic flow cytometry platform, named the “EZ DEVICE”, which automates immune cell labeling and

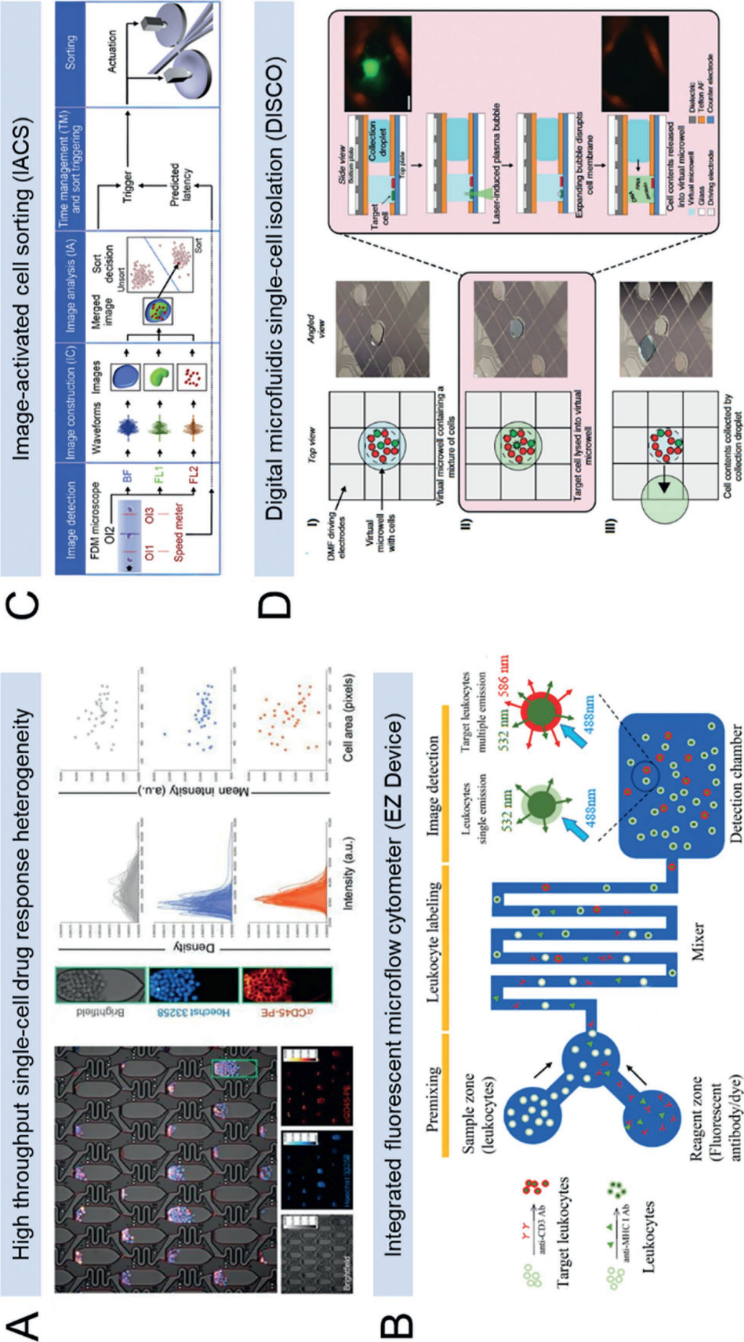


Figure 2 AI-accelerated microfluidic drug screening at single-cell level (1D). (A) Microfluidic imaging with ML enables automated phenotypic profiling and drug response quantification at the single-cell level⁷⁵. Reprinted with permission from Ref. 75. Copyright © 2021 American Association for the Advancement of Science. (B) EZ Device: combines AI-based recognition with automated sample processing and phenotyping⁷⁶. Reprinted with permission from Ref. 76. Copyright © 2024 Elsevier B.V. (C) IACS: integrates high-throughput imaging, analysis, and actuation for real-time intelligent sorting⁷⁹. Reprinted with permission from Ref. 79. Copyright © 2018 Elsevier Inc. (D) DISCO: enables laser-triggered lysis and omics-compatible content collection from selected cells⁸⁰. Reprinted with permission from Ref. 80. Copyright © 2020 Springer Nature.

Table 2 Representative AI-enhanced microfluidic technologies for drug screening.

Dimension	Target	Technique	AI Function	Application	Accessibility	Ref.
Single cell (1D)	Various cells	DL (Mask R-CNN), ML (t-SNE, clustering)	Quantify drug-induced heterogeneity in single cells	Screen diverse drug effects across single cells	Open access	Yellen et al., 2021 ⁷⁵
	Bacteria, yeast	YOLOv4 (deep CNN)	Classify real-time fluorescence and scattering signals	Rapid microbial detection at point-of-care sites	Proprietary system	Kuo et al., 2025 ⁷⁶
	Tumor, immune cells	Custom CNN	Track dynamic tumor-immune cell interactions over time	Study immune-oncology drug response mechanisms	Proprietary system	Majumder et al., 2021 ⁸²
	Blood cells	AI-guided matrix analysis	Analyze single-cell electrical impedance signatures	Assess cell states, diseases, and drug effects	Proprietary system	Caselli et al., 2022 ⁸³
	Glioblastoma cells	CNN, RNN (bi-LSTM)	Track cell migration under electric fields	Screen anti-migratory compounds for glioblastoma	Restricted access	Tsai et al., 2020 ⁸⁴
Multi-cell (2D)	Various cells	ML	Real-time image capture and sorting precision	High-throughput, image-guided cell isolation	Proprietary system	Nitta et al., 2018 ⁷⁹
	Various cells	CNN (deep)	Enhanced multi-parametric image-based cell sorting	Increase sorting speed and accuracy	Proprietary system	Isozaki et al., 2020 ⁸⁵
	Microbial cells	CNN + classical algorithm	Index-based single-cell tracking and sorting	Construct microbial strain libraries efficiently	Open access	Diao et al., 2022 ⁸⁷
	Various cells	DCNN (YOLOv5)	Isolate cells for downstream omics analysis	Enable high-resolution omics-level research	Open access	Lamanna et al., 2020 ⁸⁰
	GBM on-chip	CNN	Predict glioblastoma evolution parameters from images	Guide targeted therapy development	Proprietary system	Pérez-Aliacar et al., 2021 ¹⁰¹
	Droplet bacteria	CNN	Segment bacteria droplets in real time	Screen antibiotic susceptibility efficiently	Restricted access	Riti et al., 2024 ¹⁰²
	Bacteria	Mask R-CNN (Detectron2)	Track bacteria density changes over time	Screen complex antibiotic combinations	Proprietary system	Yang et al., 2023 ⁹⁵
	Leukemia samples	Deep convolutional autoencoder	Extract drug-induced morphology patterns	Whole-blood drug susceptibility analysis	Proprietary system	Kobayashi et al., 2019 ⁹⁹
	Blood cells	ML (k-means)	Cluster blood cell image features	Analyze drug response and thrombosis risk	Open access	Fay et al., 2023 ¹⁰³
	PDAC + CAFs	MLP neural network + SVM	Template multiparametric impedance signals	Detect drug-resistant cancer subpopulations	Proprietary system	Jarmoshti et al., 2025 ¹⁰⁴
Spheroid (3D)	Skin co-culture	SVM + PCA	Integrate multi-cell response metrics	Predict adverse drug reactions	Proprietary system	Chong et al., 2022 ¹⁰⁶
	BT474, MCF-7	AnaSP, ReViSP	Analyze 2D/3D spheroid morphology features	Optimize tumor spheroid drug evaluation	Restricted access	Lee et al., 2024 ¹²⁹
	MCF-7, HEK293FT	CNN	Classify droplets containing spheroids	Enrich spheroids for drug testing	Proprietary system	Anagnostidis et al., 2020 ¹³⁰
	HEK293FT	CNN + YOLOv4	Detect stiffness gradient influence	Study mechanical cues on growth	Open access	Anagnostidis et al., 2024 ¹³²
	SMC, HEK spheroids	Mask R-CNN	Segment images, quantify contraction areas	Assess contraction, evaluate drug effects	Open access	Akshay et al., 2022 ¹³⁴
	SUM159	CNN	Predict IC ₅₀ , viability non-destructively	Screen anticancer drugs efficiently	Proprietary system	Zhang et al., 2019 ¹⁴⁴

(continued on next page)

Table 2 (continued)

Dimension	Target	Technique	AI Function	Application	Accessibility	Ref.
Organ-on-a-chip (3D+)	SUM159, SUM149, T47D	CNN	Track dynamic spheroid responses	Test drugs on 3D spheroid models	Proprietary system	Chiang et al., 2024 ¹⁴⁵
	B16F10, UN-KC6141	CNN/TIL analyzer	Score immune infiltration in spheroids	Screen cancer immunotherapy agents	Open access	Ao et al., 2022 ¹⁴⁹
	HUVEC (angiogenesis) vMPS, islet-chip	NuSeT + skeletonization	Extract vessel length, width, branch points	Quantify angiogenesis, assess inhibitors	Proprietary system	Choi et al., 2023 ¹⁹²
	Caco-2 cells	AutoKeras	Predict oxygen transport via morphology	Optimize vascular networks, transplant models	Restricted access	Tronolone et al., 2023 ¹⁸⁸
	HSAEC	PCA + SVM	Classify tight junction integrity	Monitor barrier function, permeability	Restricted access	Calogiuri et al., 2024 ¹⁸⁹
	Gut-on-chip + probiotics BrC, PDX brain-chip	ResNet CNN	Analyze cilia motion, epithelial differentiation	Assess airway function, drug impact	Restricted access	Chen et al., 2024 ¹⁷⁵
		PCA	Rank probiotic efficacy across strains	Screen probiotics for IBD treatment	Restricted access	Wu et al., 2024 ¹⁷³
		Ensemble ML	Predict brain metastasis likelihood	Guide precision oncology decisions	Proprietary system	Oliver et al., 2019 ¹⁹³
	Tumor-immune chip	RDPV	Enhance time-lapse resolution in microscopy	Improve tracking, drug response analysis	Proprietary system	Cascarano et al., 2021 ¹⁷⁶

image analysis on-chip (Fig. 2B). The system employed the YOLOv4 DL model for accurate cell recognition and segmentation, enabling immune phenotyping through multi-channel fluorescence signals. It successfully distinguished CD3⁺ and MHC I⁺ Jurkat T cell populations with a staining efficiency as high as 99.06%. Beyond its analytical performance, the platform also supports point-of-care testing, offering significant potential for personalized immune monitoring and drug development.

Real-time tracking of tumor-immune interactions. Leveraging AI-based analysis of multicolor fluorescence and bright-field imaging, Majumder et al.⁸² employed the Dexter™ platform to co-culture tumor cells with CD4, CD8, and PBMC immune cells at single-cell resolution, enabling real-time tracking of tumor-immune interactions. Functional markers such as IFN-γ, Granzyme B, and antigen uptake were dynamically monitored, while AI algorithms captured the evolution of immune synapse formation and cytotoxic responses. This strategy not only elucidated key patterns in immune activation but also revealed interpretive gaps in cross-immune profiling, underscoring the need for broader-scale validation across diverse tumor-immune contexts. By integrating with drug screening requirements, this real-time dynamic monitoring technology is poised to become a powerful tool for HTS, accelerating the development of novel immunotherapies and targeted drugs, thereby more precisely evaluating the regulatory effects of drugs on the tumor immune microenvironment and their potential therapeutic efficacy.

Cellular dielectric properties extraction and heterogeneity analysis. Caselli et al.⁸³ developed a multi-step AI workflow integrating CNN and RNN to extract intrinsic dielectric properties directly from raw impedance data streams. This approach not only resolves single-cell signals concealed within overlapping measurements but also performs real-time processing synchronized with flow dynamics, enabling applications in cellular heterogeneity profiling and high-throughput drug screening. Such capabilities are instrumental for timely evaluation of disease progression and therapeutic responses at the single-cell level. Complementarily, Tsai et al.⁸⁴ introduced a hybrid multi-electric-field chip (HMEFC) to generate electrical stimulation gradients combined with multi-condition co-cultures. Employing automated tracking and ML algorithms, they quantitatively extracted features including cell migration directionality, electrotactic alignment, and velocity at high throughput. Their findings implicate voltage-gated calcium/potassium channels and acid-sensing sodium channels in electrotactic migration, offering novel insights into tumor heterogeneity, electric field-driven invasiveness, and anti-cancer drug target identification within precisely controlled microenvironments.

Collectively, these studies highlight the emerging synergy between AI and microfluidic platforms in single-cell drug screening, particularly in the high-throughput analysis of complex data. Fig. 3 outlines an integrated pipeline of these studies. By coupling microfluidic single-cell platforms, advanced imaging, signal processing and AI data analysis, this synergistic approach facilitates scalable drug screening and opens new avenues for translational research.

2.3.2. AI for enhanced single-cell sorting

Intelligent image-activated cell sorting (IACS) platform. Nitta et al.⁷⁹ pioneered an integrated approach by combining frequency-division-multiplexed (FDM) microscopy, a hybrid image processing architecture (FPGA + CPU + GPU), and real-time mechanical sorting into an IACS platform (Fig. 2C). By

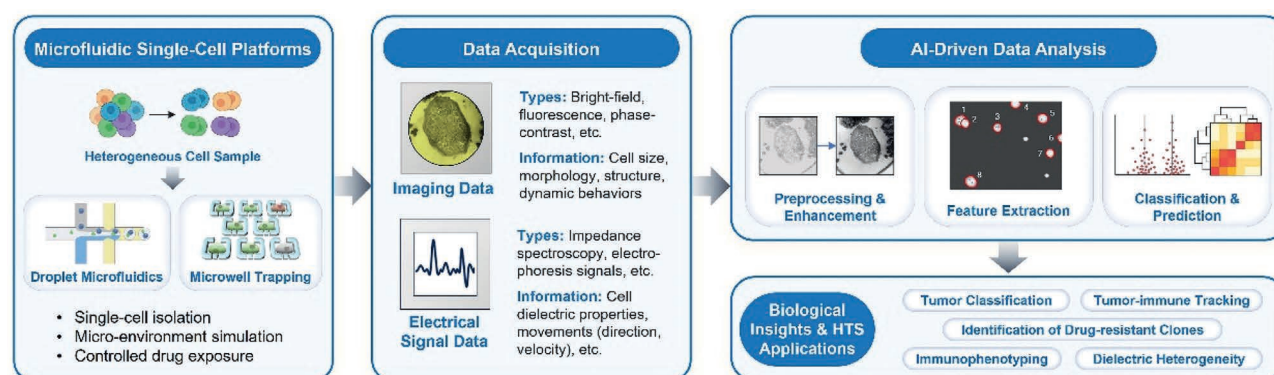


Figure 3 Schematic workflow of AI-accelerated microfluidic platforms for single-cell phenotypic analysis. This figure is created with BioRender.com.

incorporating AI, the system enables fully automated, real-time operations including data acquisition, processing, decision-making, and actuation, achieving a processing rate of 100 cells per second with an individual image analysis time below 32 ms. The platform demonstrated successful sorting of activated platelet aggregates and rare EpCAM⁺ circulating tumor cells from cancer patient blood samples, and can be seamlessly integrated with single-cell omics and drug screening workflows. Building on this work, the team further introduced the virtual-freezing fluorescence imaging technique, enabling blur-free imaging of rapidly flowing cells and increasing the sorting rate by a factor of 20 (from 100 to 2000 cells/s)⁸⁵. A parallel processing framework consisting of 8 computers and GPU clusters was implemented to further enhance image analysis throughput. Additionally, an improved CNN allowed high-dimensional learning and classification of multi-modal image features. This broadened the platform's applicability to diverse biological samples, including yeast, human cells, and organoids, thus supporting its use in practical drug screening and high-throughput functional research. Subsequently, Zhao et al.⁸⁶ incorporated a ML algorithm to improve prediction accuracy for sorting time, leading to enhanced sorting efficiency, yield, and purity of the upgraded iACS platform.

Single-cell identification and sorting of microbial samples. Diao et al.⁸⁷ developed an AI-assisted single-cell indexing and sorting system for microbial samples (EasySort AUTO), which enables the identification and isolation of individual cells directly from *in situ* specimens. Target cells are recognized by image-based algorithms under bright-field microscopy and subsequently manipulated via optical tweezers, offering significant potential for high-throughput drug screening or precise evaluation of drug performance on specific microbial populations. EasySort AUTO system demonstrates high compatibility with diverse microbial morphologies and sizes, achieving a recognition accuracy of 80%. It is well-suited for distinguishing between different microbial species and supports downstream analyses such as single-cell culturing, internal transcribed spacer sequencing, and multiple displacement amplification.

AI-enabled digital microfluidics for single-cell multi-omics. Lamanna et al.⁸⁰ introduced the Digital microfluidic Isolation of Single Cells for Omics (DISCO) platform (Fig. 2D), which integrates digital microfluidics, laser-mediated cell lysis, and AI-based image analysis for targeted single-cell selection from heterogeneous populations and subsequent multi-omics profiling. A customized CNN is employed for cell segmentation and localization, delivering strong performance (AUC = 0.991,

accuracy = 0.84) and enabling a fully automated lysis workflow. By linking imaging phenotypes (*e.g.*, fluorescence staining) with genomic, transcriptomic, and proteomic data, the platform establishes phenotype–genotype connections. It can detect genetic alterations, including CRISPR-induced deletions, and correlate them with protein expression patterns, offering valuable potential for in-depth analysis of context-dependent rare cell populations. This capability is particularly advantageous for drug screening, as it allows for the precise evaluation of drug effects on diverse cellular phenotypes and their underlying multi-omic responses, thereby facilitating the discovery of novel drug targets and personalized therapeutic strategies.

These studies demonstrate that AI-integrated microfluidic platforms enable precise, high-throughput isolation, identification and sorting of single cells. By linking phenotypic imaging with multi-omics data, they offer powerful tools for dissecting cellular heterogeneity and resistance mechanisms in drug screening and therapeutic development.

3. AI-powered microfluidic drug screening in multicellular models (2D)

3.1. Microfluidics for multicellular analysis

Population-level analysis remains a foundational strategy in cell and microbial culture-based research, particularly in pharmacological screening and systems-level biology. By capturing the collective behavior of cell populations, this approach enables robust characterization of intercellular interactions, group-level dynamics, and treatment responses across diverse conditions. Compared to single-cell methods, multicellular models are generally more accessible, cost-effective, and technically established, making them highly suitable for routine applications such as drug efficacy testing, toxicity profiling, and disease modeling. Within this context, microfluidic platforms have become increasingly prominent due to their capacity to replicate key aspects of the *in vivo* microenvironment with high spatial and temporal precision^{88–90}. Their fine-tuned control over environmental parameters, such as nutrient gradients, mechanical cues, and molecular diffusion, supports more physiologically relevant experimental models^{91,92}. The integration of multi-channel architectures further facilitates combinatorial drug testing with enhanced parallelization, consistency, and scalability. In addition, microfluidics enables systematic investigation of complex cellular phenomena at the

population level, including coordinated responses, immune signaling, and microenvironment-mediated modulation⁹³. As these systems become more entrenched in high-throughput workflows, they also contribute to the broader push toward standardization and integration in pharmacological assays.

3.2. *AI methods developed for microfluidics-based multicellular drug screening*

The rapid accumulation of high-content, multimodal datasets presents significant challenges for conventional analytical frameworks, underscoring the need for more effective strategies to fully leverage the rich data produced by microfluidic platforms. AI offers a powerful solution in this context, particularly for handling imaging and multiparametric data.

In HTS involving multicellular systems, CNNs have emerged as an effective solution for the challenges posed by large volumes of complex imaging data generated by microfluidic platforms²⁵. Due to their architecture incorporating local connectivity, parameter sharing, and translational invariance, CNNs are particularly well-suited for image recognition tasks. These models automatically learn relevant spatial features from raw data, eliminating the need for manual feature engineering and enhancing scalability across heterogeneous datasets. Although primarily focused on diagnostic applications, the use of machine vision in detection platforms, such as the CEA assay system, demonstrates AI's adaptability for rapid, image-based analysis in microfluidic contexts⁹⁴. When integrated with microfluidic multicellular assays, CNNs capture local patterns, hierarchical structures, and nonlinear relationships within complex biological images, enabling rapid, label-free, and data-driven analysis⁴⁴. This synergy significantly improves the efficiency of pharmacological evaluation, especially in handling multiplexed drug combinations and assessing dose–response effects. For example, Yang et al.⁹⁵ developed an AI framework called HTCDES, capable of programmably generating droplets containing target bacteria and diverse antibiotic combinations in a high-throughput fashion. By integrating Mask R-CNN for efficient object detection and instance segmentation, the system analyzed over 7000 brightfield droplet images with an accuracy of $95.0 \pm 5.8\%$, demonstrating its potential for rapid, systematic, and scalable drug combination screening. By overcoming the throughput limitations of conventional analysis, CNN-assisted pipelines not only accelerate drug discovery workflows but also contribute to the standardization and automation of multicellular screening processes⁹⁶.

In microfluidic multicellular assays, the acquisition of large-scale, high-dimensional data poses significant challenges for conventional analysis pipelines. With its capabilities in automatic feature extraction, nonlinear modeling, computational scalability, and adaptability, ML has emerged as a powerful approach for multiparametric data interpretation¹⁶. By integrating diverse data types generated from microfluidic platforms, ML enables precise identification of cellular response patterns, facilitating robust evaluation of drug synergy and potential toxicity. ML models can process heterogeneous data from various sources, including imaging, electrical signals, and other sensors, leading to a more comprehensive analysis of cellular behavior⁹⁷.

Different AI architectures are uniquely suited for distinct types of data and analytical challenges encountered in microfluidic drug screening⁹⁸. CNNs, with their localized connectivity and shared parameters, are best suited for extracting spatial features from static images. RNNs, including variants such as LSTM (Long

Short-Term Memory networks), are specifically designed for sequential data and are thus optimal for tracking dynamic responses or temporal cellular behaviors. Graph Neural Networks (GNNs) excel at modeling spatial relationships and structured biological networks, enabling finer interpretation of cell–cell interactions and tissue-level organization. Leveraging these diverse architectures in a complementary manner could substantially enrich the analytical depth of AI-driven evaluations, particularly in decoding complex drug responses within multicellular microenvironments.

However, the application of advanced AI models in multicellular microfluidic assays is not without its limitations. Firstly, the inherent complexity of multicellular interactions and microenvironmental variability can lead to difficulties in model generalizability, where models trained on one specific multicellular system may not perform robustly on different systems or conditions. Secondly, similar to challenges at the single-cell level, DL models often operate as “black boxes” making it challenging to interpret the precise biological mechanisms underlying drug responses or emergent collective behaviors in multicellular contexts. This lack of transparency not only can hinder the translation of AI findings into actionable biological insights, but also makes it particularly difficult to effectively decouple complex intercellular interactions and their precise contributions to drug responses in multicellular settings. Lastly, the generation and processing of large-scale, high-dimensional time-lapse data from multicellular assay, essential for capturing dynamic processes like cell migration or tissue remodeling, impose significant computational demands, requiring substantial computational infrastructure and optimization for real-time analysis. Addressing these challenges is crucial for the broader adoption and full exploitation of AI in multicellular drug screening.

3.3. *Applications of AI-assisted microfluidic multicellular drug screening*

AI applications in microfluidic multicellular drug screening encompass both image analysis and multiparametric data interpretation, significantly enhancing screening efficiency and depth. Specifically, CNNs are primarily utilized for image analysis tasks to identify cell morphology and behavior patterns due to their advantages in processing spatial data. Meanwhile, ML algorithms are more adept at integrating and interpreting multi-parametric heterogeneous data, thereby enabling a comprehensive evaluation of overall cellular behavior. Fig. 4 illustrates key platforms and methodologies leveraging AI for image analysis and multiparametric data interpretation in multicellular assays. And key examples of 2D multicellular microfluidic systems enhanced by AI are listed in Table 2.

3.3.1. *CNN-based image analysis*

In microfluidic multicellular drug screening, CNN-based image analysis can be broadly categorized into two main modes: unsupervised and supervised approaches. Supervised CNNs typically necessitate substantial amounts of pre-labeled data for training, enabling them to learn image features for classification or regression tasks. Conversely, unsupervised CNNs do not require labeled data; instead, they aim to discover patterns or perform clustering by learning the intrinsic structure of the data, which can be particularly advantageous when labeled data is insufficient.

Unsupervised CNN-based analysis. To overcome the reliance of conventional CNNs on large manually annotated datasets, focus

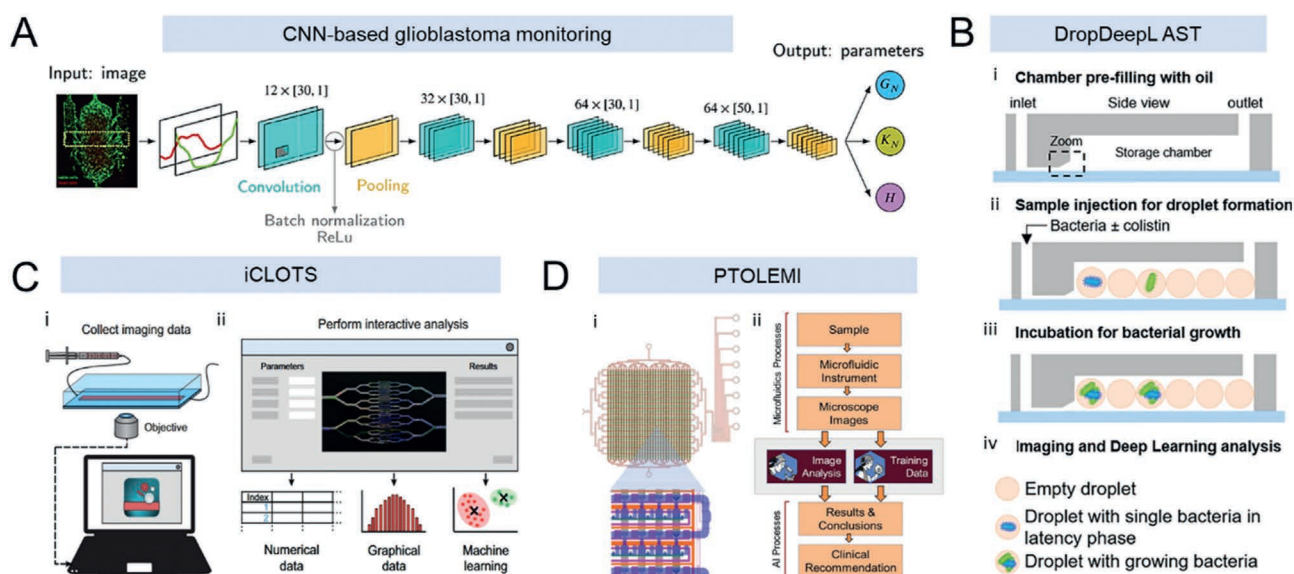


Figure 4 AI-enabled microfluidic drug screening in 2D multicellular models. (A) CNN-based glioblastoma (GBM) monitoring: CNNs applied to fluorescence images to extract cell culture parameters and predict GBM progression dynamics¹⁰¹. Reprinted with permission from Ref. 101. Copyright © 2021 Elsevier Ltd. (B) DropDeepL AST: a droplet microfluidics platform combining bright-field imaging and DL for rapid and precise antibiotic susceptibility testing¹⁰². Reprinted with permission from Ref. 102. Copyright © 2024 Elsevier B.V. (C) iCLOTS: an open-source image analysis platform integrating single-cell tracking and ML for dynamic, quantitative assessment of physiologically relevant microfluidic assays¹⁰³. Reprinted with permission from Ref. 103. Copyright © 2023 Springer Nature. (D) PTOLEMI: a Python-based tensor tumor locator integrated with microfluidic instrumentation, combining ML algorithms for rapid and accurate identification of cell viability and classification of cell types within samples¹⁰⁸. Reprinted with permission from Ref. 108. Copyright © 2023 MDPI.

has shifted to label-free learning strategies, Kobayashi et al.⁹⁹ employed a high-throughput full-blood imaging flow cytometer (processing over 10^6 cells/s) in conjunction with a deep convolutional autoencoder for label-free detection. By analyzing morphological changes in blood cells after drug treatment, the model successfully extracted drug susceptibility information. The study further validated the system's performance in detecting drug-resistant cell lines (K562/ADM) as well as clinical acute lymphoblastic leukemia samples, demonstrating high classification accuracy. It also enabled quantitative analysis of the proportion of drug-resistant cells within heterogeneous populations, effectively replacing conventional 72-h viability assays. This significantly reduced the testing time to within 24 h and provides a novel approach for constructing personalized drug sensitivity databases and performing label-free liquid biopsy. Similarly, Wu et al.¹⁰⁰ proposed a high-throughput, high-accuracy frequency-shift optical time-stretch quantitative phase imaging system. They trained a CNN constructed with autoencoders using more than 30,000 images, enabling label-free monitoring and evaluation of drug efficacy in leukemia cells with an average classification accuracy of 96.2%.

Supervised CNN-based analysis. Pérez-Aliacar et al.¹⁰¹ applied CNN to fluorescent micrographs obtained from microfluidic platforms simulating necrotic core formation in glioblastoma (Fig. 4A). The model accurately predicted the cell behavior parameters (proliferation rate, chemotaxis coefficient, hypoxia threshold) of glioblastoma cells switching between migration-proliferation behavior under hypoxic conditions, with a Pearson correlation coefficient exceeding 0.99 and RMSE below 0.1, indicating excellent predictive performance and generalizability. Notably, the CNN maintained robust accuracy even when trained on noisy input data, thereby offering an intelligent and robust

approach for personalized tumor modeling and rapid pharmacodynamic assessment. Riti et al.¹⁰² trained and validated a CNN using a dataset of 92,000 individual droplet images to enable rapid antibiotic susceptibility testing on a droplet-based microfluidic platform (Fig. 4B). The system supports parallel analysis of over 3000 droplets and accurately classifies susceptible and resistant bacterial strains within 2 h using label-free bright-field imaging, achieving 100% concordance with standard reference methods.

3.3.2. ML-based multiparametric analysis

Characterization of cell behavior and properties. ML demonstrates strong capabilities in integrating and interpreting large-scale, high-dimensional multiparametric data generated by microfluidic multicellular assays. For example, Fay et al.¹⁰³ developed iCLOTS (Interactive Cellular assay Labeled Observation and Tracking Software) for microfluidic live-cell imaging experiments (Fig. 4C). It translates microscopy data into high-dimensional quantitative datasets, accounting for cellular heterogeneity and dynamics under flow conditions. The tool integrates traditional image processing with ML algorithms, enhancing throughput and analytical accuracy for drug discovery workflows. Jarmoshti et al.¹⁰⁴ employed a microfluidic ultra-parabolic extensional flow field to achieve contact-free, high-throughput (~ 100 cells/s), clog-free cellular deformability measurements. In addition to capturing impedance amplitude and pulse width, their system incorporated frequency-dependent responses, such as phase shift, amplitude, and impedance opacity at 0.5 and 18 MHz, thereby mitigating errors associated with shape-based measurements alone. A multilayer perceptron (MLP) was used to model complex nonlinear relationships, while a SVM performed the final classification. This approach successfully distinguished viable cancer cells from cancer-associated fibroblasts, offering a

powerful tool for screening candidate therapeutics targeting drug-resistant and metastatic cancer cells. Mencattini et al.¹⁰⁵ developed a microfluidic platform integrated with ML to evaluate red blood cell (RBC) mechanical responses under pharmacological conditions in pyruvate kinase deficiency models. The system captures dynamic RBC deformation profiles as cells traverse micropillar arrays, extracting morphological features to train a classifier capable of distinguishing treatment-modulated versus untreated populations. This label-free approach enables high-throughput assessment of drug-induced biomechanical alterations, offering a functional readout for evaluating therapeutic efficacy in hematological disorders.

Assessment of drug toxicity and efficacy. Chong et al.¹⁰⁶ developed a four-chamber microfluidic co-culture array capable of simultaneously culturing hepatic, immune, epidermal, and dermal cells. The platform generates five distinct biological readouts from each drug testing cycle and integrates these multi-parametric data to classify the skin sensitization potential of compounds using a SVM, with principal component analysis (PCA) employed for data visualization. The system achieved 100% sensitivity, 87.5% accuracy, and 75% specificity across a panel of 11 FDA-labeled compounds. Notably, it prospectively predicted the skin sensitization potential of obeticholic acid, consistent with reported clinical adverse effects, offering a novel approach for preclinical drug safety assessment. Badiola-Mateos et al.¹⁰⁷ developed a multi-layered microfluidic platform integrating a multi-frequency *trans*-endothelial electrical resistance (MTEER) sensor array designed to monitor the integrity of blood–brain barrier (BBB) models. By applying impedance spectroscopy across a broad frequency range, the system quantitatively captured changes in barrier function under various physiological and pharmacological conditions. Machine learning algorithms were employed to classify TEER signatures corresponding to distinct barrier states, enabling accurate discrimination between intact, disrupted, and recovering BBB layers. This approach enhanced the sensitivity and resolution of TEER-based assessments and offers a robust tool for evaluating neurovascular responses to drug exposure *in vitro*. Moreover, Moerdler et al.¹⁰⁸ developed PTOLEMI (Personalized Cancer Treatment through Machine Learning-Enabled Image Analysis of Microfluidic Assays), a high-throughput image analysis platform enhanced with ML, capable of rapidly processing large datasets with high accuracy (Fig. 4D). This system enables fine-grained insights into cell dynamics, allowing rapid discrimination of cell viability and subtypes across complex samples. It provides an efficient means of assessing therapeutic efficacy and supports the development of personalized treatment strategies in clinical contexts.

Collectively, these advances highlight the transformative potential of AI, particularly CNNs and ML, in microfluidic multi-cellular drug screening. By enabling scalable, multiparametric analysis of complex biological data, AI not only improves the accuracy and throughput of drug evaluation but also paves the way for more systematic, data-driven discovery of therapeutic strategies.

4. AI-accelerated microfluidics-based spheroid (3D) drug screening

4.1. Microfluidic 3D spheroid systems for drug testing

Spheroids are 3D cellular aggregates formed through self-assembly of thousands of cells¹⁰⁹. These artificially constructed *in vitro* structures closely mimic the physiological properties of

in vivo tissues, including cellular metabolism, proliferation, and concentration gradients. As a result, spheroids can more accurately reproduce complex biological phenomena such as cell–cell interactions, matrix deposition, microenvironmental cues, and physiological gradients of flow, oxygen, and nutrients, thereby offering enhanced predictive power in drug response evaluation¹¹⁰. This is especially valuable in cancer drug screening, where traditional 2D monolayer cultures fail to replicate key tumor characteristics such as cell–cell contact and the native tumor microenvironment, often resulting in attenuated malignant phenotypes compared to actual tumors¹¹¹. In contrast, 3D spheroid models better capture the complexity of solid tumors and reproduce critical features essential for pharmacological testing, such as hypoxic conditions, extracellular matrix signaling, pH levels, nutrient accessibility, and drug penetration, overcoming key limitations of conventional culture methods¹¹².

Traditional spheroid generation methods include the hanging drop technique, scaffold-based systems, and hydrogel approaches¹¹³. While these approaches remain widely used, they are often limited by low throughput, labor-intensive procedures, and challenges in maintaining consistent spheroid size, performing medium exchange, and minimizing cross-contamination¹¹⁴. Microfluidic-based spheroid culture addresses many of these limitations by enabling perfusion-based cultivation, which ensures stable nutrient supply and efficient waste removal while simulating *in vivo*-like dynamic conditions¹¹⁵. Additionally, it allows for parallel spheroid culture and drug screening, thereby improving experimental throughput and reproducibility¹¹⁶. Emerging techniques like droplet microfluidics further enhance this approach by offering high-throughput single-cell encapsulation, multifunctional droplet templating, and streamlined integration of multiple operational steps. Nowadays, microfluidic spheroid platforms have been widely adopted to model tumor microenvironments^{117,118}, dynamically monitor cellular behaviors^{119–121}, as well as evaluate both cytotoxicity and pharmacological efficacy of candidate drugs^{122–125}, with particular promise for applications in personalized cancer therapy^{126,127}. These advancements underscore the utility of microfluidic spheroid systems for drug testing. While microfluidics offers significant advantages, the precise control and optimization required for spheroid production and culture remain challenging. Recognizing this, the following section highlights how AI methodologies provide sophisticated solutions that revolutionize spheroid construction, enabling refined regulation of formation conditions and microenvironmental optimization. Fig. 5 highlights the integration of AI with microfluidic platforms for 3D cell culture analysis, including spheroid modeling, microbead generation, and immunotherapy assessment. These systems leverage DL algorithms to automate real-time classification, viability prediction, and droplet selection under complex 3D conditions. And representative applications of AI in spheroid-based drug screening platforms are outlined in Table 2.

4.2. AI methodology in microfluidic spheroid production and culture

The deep integration of AI and microfluidic technologies has revolutionized the construction of spheroid models, offering an advanced platform for precisely regulating spheroid formation conditions and optimizing their microenvironments. This integration primarily manifests in several key applications of AI methodologies.

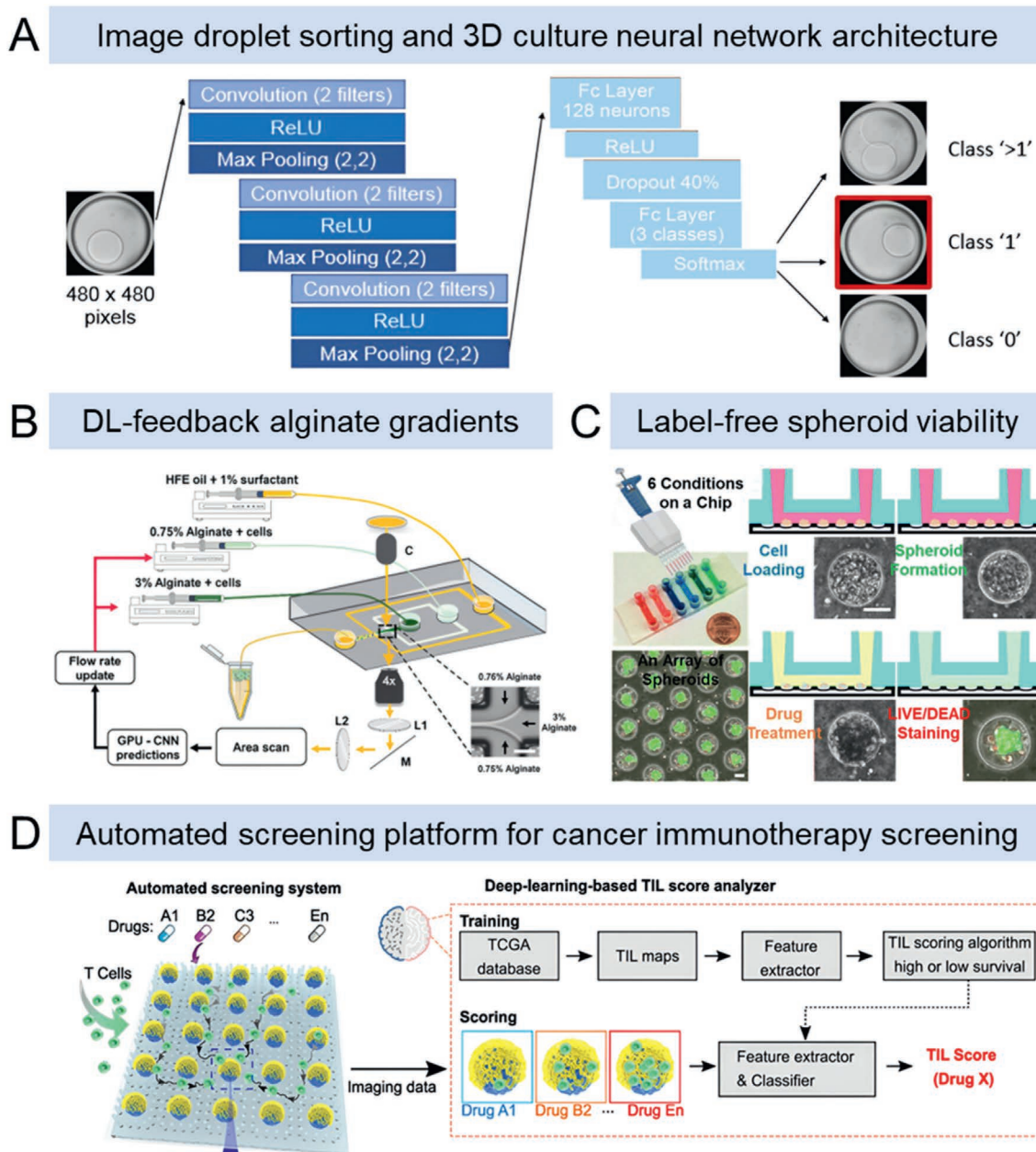


Figure 5 AI-integrated microfluidic platforms for 3D cell culture analysis and screening. (A) A deep neural network enabling real-time, accurate classification of single droplet images based on the presence and number of micro-objects¹³⁰. Reprinted with permission from Ref. 130. Copyright © 2020 Royal Society of Chemistry. (B) A droplet microfluidics platform utilizing CNN-based real-time flow rate adjustment to generate alginate microbeads, combined with YOLOv4-based selection of droplets¹³². Reprinted with permission from Ref. 132. Copyright © 2024 Frontiers Media S.A. (C) A cancer spheroid microfluidic platform integrated with a DL model for non-destructive, label-free viability estimation from phase-contrast images to monitor dynamic viability changes during drug treatment¹⁴⁵. Reprinted with permission from Ref. 145. Copyright © 2024 Royal Society of Chemistry. (D) An automated high-throughput microfluidic platform using a clinical data-driven deep learning TIL score analyzer to evaluate the therapeutic efficacy of different treatments¹⁴⁹. Reprinted with permission from Ref. 149. Copyright © 2022 National Academy of Sciences.

Firstly, in the domain of intelligent control over spheroid assembly parameters, AI has enabled the optimization of high-throughput production workflows. For instance, Trossbach et al.¹²⁸ established a DL-based workflow in which CNNs were employed for the intelligent morphological classification of cell aggregates. This AI framework systematically optimized critical parameters (*e.g.*, surfactant concentration and incubation time) for

three tumor cell lines formed *via* droplet-based microfluidics, significantly improving both assembly efficiency and morphological uniformity. This demonstrates AI's capacity to learn from complex experimental data and guide improvements in production conditions.

Secondly, for the automated analysis and optimization of spheroid morphological features, AI tools have substantially

enhanced the reliability of drug response assessment. Lee et al.¹²⁹ introduced AI-based tools (AnaSP and ReViSP) designed for automated morphological feature extraction and 3D reconstruction of cellular spheroids. Through AI model analysis of key parameters such as spheroid compactness, circularity, and necrotic core formation, researchers could optimize the initial spheroid diameter and the timing of drug screening. This effectively mitigated biases in drug response assessment caused by spheroid size variation and necrotic core formation, thereby significantly improving the reliability of high-throughput drug testing on spheroid-based platforms.

Furthermore, AI plays a crucial role in the real-time screening and enrichment of high-quality spheroids. Anagnostidis et al.¹³⁰ achieved real-time classification of individual droplet images containing multicellular spheroids by training neural networks on a large, manually curated image dataset (Fig. 5A). This AI capability, integrated with image-based sorting, allowed for the selective enrichment of high-quality, proliferative spheroids for downstream high-content imaging and drug screening. In an experiment culturing Hek293FT cells within agarose microgels, approximately 50,000 spheroids were formed, and post-screening enrichment using AI selectively raised the proportion of successful cultures demonstrating active proliferation after four days from 20% to nearly 80%. Compared to conventional platforms, this AI-driven screening approach significantly reduced the time, labor, and variability associated with spheroid generation and enrichment, providing a robust foundation for subsequent phenotypic and pharmacological analyses.

Lastly, AI has been applied to the intelligent regulation and quantification of microenvironmental mechanical properties. Given the well-established influence of microenvironmental mechanical properties on cell phenotype and drug responsiveness¹³¹, Anagnostidis et al.¹³² further coupled conventional microfluidic co-flow systems with a closed-loop, AI-guided feedback control framework to generate unsupervised stiffness gradients within hydrogel matrices (Fig. 5B). They utilized the YOLOv4 object detection algorithm to analyze time-lapse images across an 8-day culture period, developing the first quantitative model linking matrix stiffness to spheroid proliferation rates.

These AI-driven advances not only reinforce the pivotal role of the microenvironment in regulating spheroid behavior but also clearly illustrate how intelligent microfluidic systems can enable end-to-end innovation, ranging from experimental optimization to mechanistic insight, in the cultivation and utilization of 3D multicellular models through AI technologies. Building upon these advancements in spheroid production and microenvironmental control, AI further plays a pivotal role in the precise and quantitative assessment of drug efficacy on these sophisticated 3D platforms, as detailed in the subsequent section.

4.3. AI-enabled quantitative drug efficacy assessment on microfluidic spheroid platforms

The integration of AI with microfluidic spheroid platforms through the construction of intelligent analytical systems has not only significantly enhanced the efficiency and precision of spheroid production and culture workflows but also fundamentally improved the reliability of drug screening outcomes. Its core value lies in the efficient interpretation of multidimensional tumor spheroid data, demonstrating pronounced advantages particularly in high-throughput compound library screening scenarios. In the field of image processing, AI has overcome multiple technical barriers, achieving substantial progress in bright-field microscopy

image analysis^{133,134}, fluorescence microscopy image interpretation^{135–137}, mass spectrometry imaging data processing¹³⁸, and 3D image reconstruction¹³⁹, collectively forming a robust multimodal imaging analytical matrix. This section will highlight key applications of AI in quantitative drug efficacy assessment, specifically focusing on spheroid detection and segmentation, label-free spheroid viability assessment, and immune cell tracking and drug discovery.

Spheroid detection and segmentation. Notably, the research team led by Akshay developed a DL framework named SpheroScan, which utilizes the Mask R-CNN architecture to enable precise detection and instance segmentation of 3D spheroids¹³⁴. Their training dataset encompasses diverse experimental conditions, including variations in illumination intensity, culture media composition, and cell line types, and has undergone iterative optimization *via* manual annotation to enhance data quality. SpheroScan exhibits excellent cross-scenario adaptability, achieving recognition accuracies of 0.927 and 0.899 on IncuCyte imaging and conventional microscopy datasets, respectively. Additionally, the algorithm demonstrates strong potential for industrial deployment, with a per-frame processing time below 1 s and a linear computational complexity that supports large-scale image analysis. Validation across multiple datasets confirmed the model's ability to successfully identify over 90% of spheroid samples, underscoring its generalizability. Based on a variety of existing spheroid analysis platforms such as MISpheroID¹⁴⁰, SMART¹⁴¹, and SpheroidAnalyseR¹⁴², the systematic integration of DL-powered multidimensional spheroid analysis with microfluidic spheroid culture platforms holds great promise for enabling a new generation of intelligent drug screening systems¹⁴³.

Label-free spheroid viability assessment. Zhang et al.¹⁴⁴ developed a microfluidic chip capable of generating 1920 tumor spheroids. Using traditional LIVE/DEAD staining as the ground truth for spheroid viability, they trained a CNN to estimate cell viability under treatment with three different anticancer drugs based solely on bright-field images. The predicted viability showed strong correlation with staining results ($R > 0.84$), offering a rapid, low-cost, and label-free approach for assessing tumor spheroid viability in large-scale microfluidic drug screening. However, due to limitations in the training dataset size, although the model performed well for several drugs, its generalizability to other drugs or cell types remains to be fully validated, indicating reliability concerns for cross-cell line applications. Chiang's team integrated a high-throughput microfluidic chip (12,000 microwells across 6 channels) with a label-free viability estimation DL model based on phase-contrast images (Fig. 5C)¹⁴⁵. They trained and tested the model with eight conventional chemotherapeutic agents at varying concentrations, achieving a correlation coefficient as high as 0.989 with live/dead staining results. Furthermore, the model successfully predicted the efficacy of novel compounds and different cell lines, confirming its reliability and broad applicability.

Immune cell tracking and drug discovery. AI has been extensively applied in evaluating cancer immune responses within *in vitro* spheroid platforms^{146–148}. Notably, Ao et al.¹⁴⁹ introduced an automated high-throughput microfluidic platform capable of generating size-consistent spheroid arrays allowing for free perfusion of immune cells and real-time imaging to track T cell migration, infiltration, and cytotoxic activity within 3D tumor spheroids (Fig. 5D). Utilizing a DL method driven by clinical data, they scored T cell infiltration patterns to evaluate treatment efficacy. Screening a drug library with this system led to the

identification of an epigenetic drug, LSD1i, which effectively promotes T cell tumor infiltration.

The integration of AI with microfluidic spheroid platforms has markedly advanced drug screening by enabling high-throughput, precise, and often label-free analysis of 3D tumor models. This synergy significantly accelerates the multidimensional data processing of spheroids, enhances screening efficiency, and improves the accuracy and specificity of drug evaluation^{16,150}. Consequently, these AI-empowered systems markedly boost the physiological relevance and predictive accuracy of preclinical assays, thereby laying a robust foundation for the rapid identification of promising drug candidates and the development of personalized therapeutics and precision medicine.

5. AI-enhanced Organ-on-a-chip (OoC) drug screening

5.1. Organoids and Organ-on-a-chip systems

The rapid evolution of microfabrication technologies and tissue engineering has collectively ushered in a new era of *in vitro* models, with organoids emerging as an innovative class of 3D cell cultures¹⁵¹. Organoids are self-assembling 3D cellular aggregates derived from stem cells (including pluripotent or adult stem cells) or primary tissues *in vitro*^{152,153}, which stably retain and mimic key histological and functional features of their corresponding organs, including organ-specific cell types, regional differentiation, cellular organization, and partial functions of the original organ¹⁵⁴. Compared to traditional 2D cell cultures and simpler 3D spheroids, organoids can more accurately replicate the complexity of *in vivo* tissues, maintaining high fidelity in gene expression and morphological characteristics similar to the original tissue, thereby providing more physiologically relevant models for drug screening. They are better equipped to simulate drug penetration, distribution, and metabolism within tissues, and to reflect tissue-specific cellular responses, offering significant advantages in predicting drug efficacy and toxicity, particularly in complex disease models such as cancer, genetic disorders, and infectious diseases.

Building upon the advancements in organoids, Organ-on-a-chip (OoC) systems further integrate microfluidics with tissue engineering to construct microengineered biomimetic systems. An OoC is a multi-channel 3D microfluidic cell culture device designed to precisely recapitulate organ-specific physiology and functions^{152,153}. Unlike traditional microfluidic systems or static organoid cultures, OoCs incorporate dynamic fluid flow, mechanical forces, electrical stimuli, and gradients for nutrient and waste exchange, faithfully mimicking the *in vivo* microenvironment, including vascular perfusion and interstitial flow. These dynamic conditions are crucial for accurately simulating drug absorption, distribution, metabolism, and excretion (ADME) processes¹⁵⁵. OoCs have been successfully developed to model a wide range of human organs, such as heart¹⁵⁶, liver¹⁵⁷, intestine¹⁵⁸, joint¹⁵⁹, skin^{160,161}, cochlea¹⁶², and uterus^{163–165}, significantly enhancing the physiological relevance of *in vitro* studies for drug metabolism, transport, and barrier permeability^{166,167}. Moreover, OoCs inherently facilitate complete observation and control of the system due to their miniaturization, high integration, and low consumption, providing unique tools for long-term drug exposure studies and chronic disease models¹⁶⁸.

This high physiological relevance, combined with the capacity for long-term cell culture and real-time dynamic monitoring, positions both organoids and OoC platforms as transformative

advancements beyond traditional animal and 2D cell models. They can more accurately predict drug behavior and effects in the human body¹⁶⁹, thereby improving the success rate and de-risking candidate drugs across various stages of drug discovery, from target identification to preclinical evaluation¹⁷⁰. With the enactment of the FDA Modernization Act 2.0 (2022), which removed mandatory animal testing requirements for new drugs¹⁷¹, OoC technology has gained unprecedented impetus, establishing itself as the next-generation *in vitro* model for drug discovery and disease modeling, offering superior predictive accuracy, cost-effectiveness, and enhanced ethical compliance. Fig. 6 presents a diverse range of AI-enhanced OoC platforms, illustrating how ML and DL methods contribute to functional readouts, high-content analysis, and dynamic modeling within physiologically relevant systems. These platforms demonstrate how AI enables advanced feature extraction and interpretation in complex OoC models, bridging imaging data, biological function, and therapeutic response. And Table 2 includes selected AI-powered OoC systems used for advanced drug screening. This chapter will primarily focus on how the synergy of AI and OoC technologies is revolutionizing drug screening by substantially improving automation, predictive power, and throughput in these complex systems.

5.2. AI methodologies and challenges in OoC systems

Patient-derived OoC systems represent a highly promising class of 3D preclinical models, offering an unprecedented platform for precision medicine and drug discovery²⁰. These microphysiological systems are capable of generating incredibly rich and multidimensional data, spanning from cellular morphology, gene expression, proteomics, and metabolites to real-time physiological signals. However, to fully exploit their potential, precisely replicating the intricate physiological microenvironment presents significant technical challenges, particularly during drug screening, where efficient and accurate extraction and analysis of high-throughput, multimodal, and dynamically changing data is highly difficult¹⁷⁸. This challenge primarily stems from the inherent heterogeneity of cell populations within organ chips, their complex intercellular interactions, and the intricate spatiotemporal dynamics of biological responses⁹⁷. Effectively extracting actionable insights from such complex, high-dimensional, and often noisy datasets urgently demands advanced computational frameworks that go beyond traditional analytical methods.

AI technologies offer powerful solutions for addressing these challenges in OoC research. The optimal selection of AI methodologies, encompassing both traditional ML models such as SVM and advanced DL algorithms, is paramount and should be guided by the specific biological question, the complexity of the data, and crucially, the required level of model interpretability. AI can transcend traditional feature engineering by automatically extracting deep biological patterns from raw, multimodal OoC data¹⁷⁹ (e.g., high-content imaging, electrophysiological recordings, mechanical sensing, and integrated multi-omics data streams) through unsupervised feature learning such as autoencoders and generative adversarial networks, significantly reducing reliance on time-consuming manual annotation¹⁸⁰. This capability not only enables automated, fine-grained classification of biological features, including cellular morphology, proliferation dynamics, migration behavior, and molecular marker expression¹⁷⁰, but also facilitates real-time predictive modeling of drug-induced cellular responses, allowing for early identification of potential toxicities or efficacies. Building upon these analytical strengths,

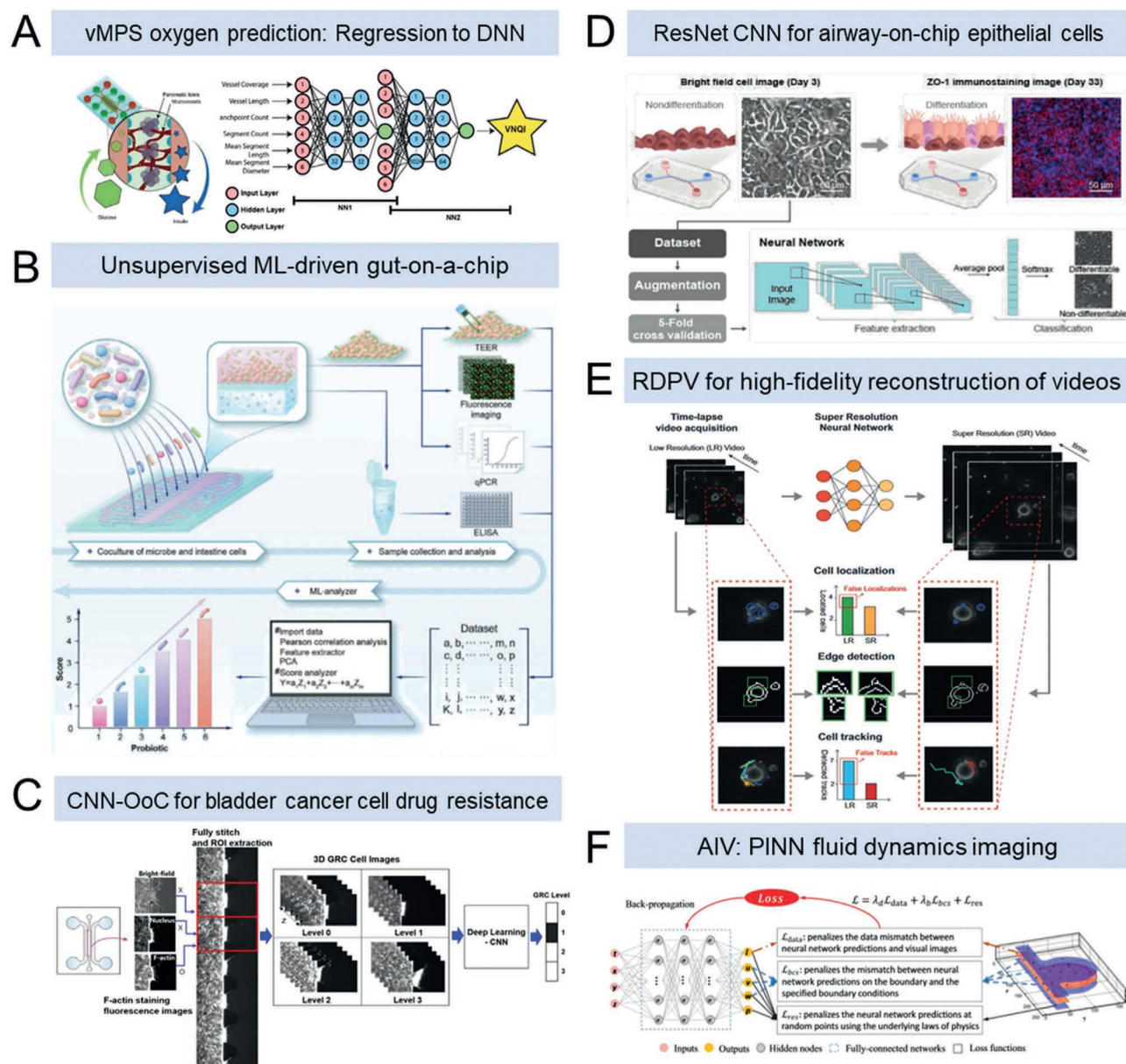


Figure 6 AI-enhanced Organ-on-a-chip drug screening. (A) A chained neural network trained on morphological metrics from vMPS to generate VNOI predictive of tissue oxygenation¹⁷². Reprinted with permission from Ref. 172. Copyright © 2023 Wiley. (B) A ML-integrated gut-on-a-chip platform with an unsupervised scoring analyzer for screening probiotic strains with anti-inflammatory efficacy¹⁷³. Reprinted with permission from Ref. 173. Copyright © 2024 Wiley-VCH GmbH. (C) An organ-on-a-chip-based validation platform employing CNNs to classify drug resistance levels in bladder cancer cells based on high-content image data¹⁷⁴. Reprinted with permission from Ref. 174. Copyright © 2024 Frontiers Media S.A. (D) A label-free morphological imaging platform compatible with small airway-on-a-chip devices, integrating DL for early prediction of HSAEC differentiation¹⁷⁵. Reprinted with permission from Ref. 175. Copyright © 2024 MDPI. (E) Recursive Deep Prior Video (RDPV) method extending DIP to time-lapse microscopy for super-resolved tracking and quantification of tumor-immune cell interactions¹⁷⁶. Reprinted with permission from Ref. 176. Copyright © 2024 Elsevier B.V. (F) AI velocimetry using physics-informed neural networks to quantify blood flow velocity and stress fields from imaging data for hemodynamic assessment in microvascular disease models¹⁷⁷. Reprinted with permission from Ref. 177. Copyright © 2021 National Academy of Sciences.

AI is increasingly enabling intelligent experimental design and closed-loop control, for instance, by utilizing reinforcement learning or Bayesian optimization algorithms to dynamically adjust fluidic conditions, cell seeding densities, or drug gradients based on real-time data feedback, thereby maximizing physiological relevance or screening efficiency and transforming OoC platforms from passive observation to efficient autonomous

manipulation¹⁸¹. The emergence of intelligent OoC (iOoC) systems, which integrate *in situ* sensors for continuous cellular and microenvironmental monitoring, is pivotal for realizing robust closed-loop control and comprehensive bioanalysis, thereby transitioning OoC platforms towards true autonomous operation^{179,182–184}. Furthermore, the advent of AI agent systems, leveraging generative models and large language models,

significantly advances this transformation by enabling sophisticated capabilities such as autonomous hypothesis generation, virtual cell simulation, and self-correcting experimental design within OoC platforms^{185,186}.

Despite AI's immense potential in empowering OoC, its widespread adoption still faces several challenges. Firstly, while OoC data is information-rich, the scarcity of high-quality labeled data and the lack of standardized, interoperable benchmark datasets severely impede AI model training and cross-platform generalizability, especially given the heterogeneity across different experimental batches and platforms. Secondly, precisely modeling complex multi-organ interactions, systemic drug effects, and long-term dynamic adaptations within integrated OoC systems poses a substantial challenge, as it requires correlating responses across distinct tissue types and mimicking dynamic physiological crosstalk. AI, through its capacity for integrating multimodal and multi-omics data, can help predict complex systemic responses and optimize drug delivery strategies in multi-organ setups¹⁸⁷. However, extracting interpretable biological mechanisms from these complex AI models, particularly regarding causal drug–action relationships in multi-organ contexts, remains a significant hurdle due to the prevalent “black-box” nature of many advanced AI models^{70,71}. To enhance transparency and derive actionable insights for drug mechanism studies, the integration of explainable AI (XAI) techniques or the preference for more interpretable models is increasingly emphasized. Thirdly, developing robust AI architectures for OoC data analysis requires continued investment. These architectures must not only effectively process multidimensional, heterogeneous, and highly time-dependent data (e.g., cell differentiation trajectories and tissue remodeling processes), but also ensure high reliability, interpretability, and reproducibility across various experimental conditions. Future research must thus focus on overcoming these limitations to fully unleash the transformative power of AI in OoC drug screening.

5.3. Applications of AI in OoC drug screening

Building upon the AI methodologies and challenges previously discussed, the following chapter will present specific applications of AI in OoC drug screening. We will primarily explore two core dimensions: first, how AI enables in-depth analysis of multidimensional, multiparametric, and multi-omics data generated by OoCs, facilitating a comprehensive understanding of complex drug effects; and second, how AI achieves real-time, continuous monitoring of cellular dynamics and physiological processes within OoCs, thereby elucidating dynamic mechanisms of drug action. These case studies will illustrate how AI significantly enhances the efficiency, depth, and predictive accuracy of OoC platforms in drug discovery and personalized medicine.

5.3.1. AI-driven comprehensive multi-modal and multi-omics data analysis

A key application of AI in OoC drug screening lies in its powerful ability to analyze complex, multidimensional data. This section will demonstrate how AI integrates morphological, functional, multiparametric, and even multi-omics data generated from OoC platforms to achieve a comprehensive, high-throughput understanding of drug effects, thereby overcoming the limitations of traditional methods.

Refined vascular network prediction. Tronolone et al.¹⁸⁸ employed REAVER software to segment and analyze vascular

network morphology, extracting key geometric features that were subsequently subjected to dimensionality reduction using PCA and factor analysis. A suite of regression models, including multivariate linear and random forest regression, was then applied to predict morphological and functional outputs of the vascularized microphysiological systems (vMPS). Although the model successfully reduced computational load and offered a framework for vascular function assessment, its predictive accuracy for oxygen transport capacity was limited ($R^2 < 0.8$), likely owing to the complexity of the underlying data and insufficient feature representation. To address these limitations, the same group advanced their methodology by implementing a chained deep neural network for the predictive modeling of oxygen delivery in vMPS (Fig. 6A)¹⁷². By systematically varying experimental parameters such as cell type, seeding density, matrix stiffness, and growth factor composition, they generated a diverse library of 500 vMPS models. Morphological descriptors (e.g., vascular coverage, total vessel length, branching complexity) were extracted from high-resolution images, and oxygen transport capacity was computed using the AngioMT software. These multimodal datasets were integrated into a chained neural network whose output, the VNQI (Vascular Network Quality Index), demonstrated exceptional concordance with experimentally measured oxygen transport values ($R^2 = 0.98$). Moreover, VNQI exhibited high sensitivity to biological and physical parameters influencing vascular architecture, thereby establishing a scalable, automated framework for the functional evaluation of engineered vascular systems.

Multimodal screening and function assessment. Beyond vascular networks, AI has proven increasingly valuable for the integration of high-content image data across a range of OoC applications. For instance, Calogiuri et al.¹⁸⁹ combined Raman microspectroscopy with ML to non-invasively characterize, in real time, structural and biochemical alterations in tight junctions of colorectal epithelial cells within an intestinal OoC model. Using PCA for feature compression and SVM classification algorithms, they accurately discriminated between degrees of epithelial barrier disruption with a classification accuracy of 91.9% using only seven spectral features. These findings were further validated by *trans*-epithelial electrical resistance measurements and immunofluorescence staining, underscoring the method's utility for probing barrier integrity *in situ*. Complementarily, Wu et al.¹⁷³ established a 16-channel gut-on-a-chip platform combined with unsupervised ML to rapidly evaluate the therapeutic efficacy of diverse probiotic strains (Fig. 6B). Using a dataset of 21 biomarkers, PCA was employed to generate a composite efficacy score. ANOVA and Dunnett's tests were then used to rank and compare 12 probiotic strains, leading to the identification of the top candidate strain (BL#3-14), which was further validated *in vivo* using a murine chronic colitis model. This platform not only provides a robust analytical pipeline for probiotic screening but also holds substantial potential for extending to more complex microbial ecosystems, including synbiotics and other live biotherapeutic modalities.

High-content image analysis for diverse OoC models. Paek et al.¹⁹⁰ developed a high-throughput, biomimetic bone-on-a-chip platform that spatially and structurally recapitulates bone tissue units, specifically osteons. They employed CNNs to analyze large-scale cellular image datasets obtained from the system, enabling accurate evaluation of a newly developed osteoporosis drug targeting sclerostin (anti-SOST antibody). The AI classifier achieved an accuracy of 99.5% and an AUC of 1.00 on datasets comprising β -catenin, nuclear, and merged channel images. By incorporating

AI-driven analytics, this platform offers a powerful and efficient tool for screening drug candidates in bone-related diseases, thus facilitating preclinical drug development in a mechanistically informed and scalable manner. Similarly, for cancer drug resistance prediction, Tak et al.¹⁷⁴ co-cultured bladder cancer cells exhibiting varying degrees of drug resistance with human umbilical vein endothelial cells (HUVECs) to generate 3D cellular architectures with distinct resistance phenotypes (Fig. 6C). High-content screening microscopy was employed to acquire cell images, followed by image stitching, cropping, and z-axis slice selection to reduce feature redundancy. A CNN was then trained on a dataset of 2674 images, with performance significantly improved through data augmentation and a stepwise learning rate decay strategy, thus effectively mitigating overfitting under small-sample conditions. The resulting model achieved a classification accuracy of 95.2%, with an average sensitivity of 90.5%, specificity of 96.8%, and AUC values exceeding 0.988 across all classes, highlighting its strong discriminative power. This framework not only enables reliable classification of cancer cells by drug resistance level but also holds promise for informing personalized therapeutic strategies.

Collectively, these advances highlight the promising potential of AI-assisted analysis in OoC research. By enabling the automated, multidimensional interrogation of complex cellular microenvironments, AI-driven platforms not only address longstanding challenges in cell-type identification and functional assessment under physiologically relevant conditions, but also accelerate the translation of OoC technologies for drug discovery, disease modeling, and personalized medicine.

5.3.2. AI-enabled real-time monitoring and dynamic assessment

The dynamic nature of OoC systems allows them to mimic complex physiological processes *in vivo*, providing a transformative platform for drug screening. AI integration significantly enhances this capability, allowing for superior capture, analysis, and prediction of these dynamic changes. Specifically, advanced AI technologies,

such as DL algorithms, facilitate the efficient and precise extraction and real-time interpretation of complex, temporally resolved data from OoCs' physiologically relevant 3D architectures, including biomimetic cellular organization, tissue microenvironments, and perfusable vascular networks, thereby revealing the dynamic mechanisms of drug action¹⁸¹. Fig. 7 gives a workflow for AI-enhanced dynamic analysis within OoC systems.

High-throughput quantification of angiogenesis. Vasculogenesis-on-a-chip constitutes an essential functional component of OoC platforms, facilitating nutrient delivery, waste removal, homeostatic regulation, and the structural organization of engineered tissues and organs¹⁹¹. However, the accurate quantitative characterization of diverse and structurally complex vascular phenotypes within these micro-physiological systems has consistently remained a substantial technical hurdle. To address this, Choi et al.¹⁹² developed a deep learning (DL)-enabled image processing pipeline for the high-throughput analysis and quantification of on-chip angiogenesis. Their approach integrates vascular skeletonization for structural feature extraction with a U-Net-based NuSeT algorithm for accurate nuclear segmentation, thereby translating intricate 3D vascular networks into a set of 16 quantifiable parameters, including vessel length, diameter, network density, and nuclear distribution. By incorporating multimodal analytical techniques, the pipeline enables robust temporal and spatial tracking of angiogenic dynamics. Notably, this method quantifies structural changes in angiogenesis within 2–3 min and successfully reveals the mechanisms by which growth factors such as VEGF and S1P influence vascular formation. Overcoming the subjectivity and low throughput of manual analysis tools like ImageJ and CellProfiler, it offers a rapid and accurate approach for pathological angiogenesis research and drug development.

Cell differentiation and functional prediction. Chen et al.¹⁷⁵ employed a DL-based CNN, specifically the ResNet architecture, to predict the differentiation trajectory of epithelial cells in a small airway-on-chip system (Fig. 6D). By analyzing phase-contrast microscopy images acquired on Day 3 of culture, the model

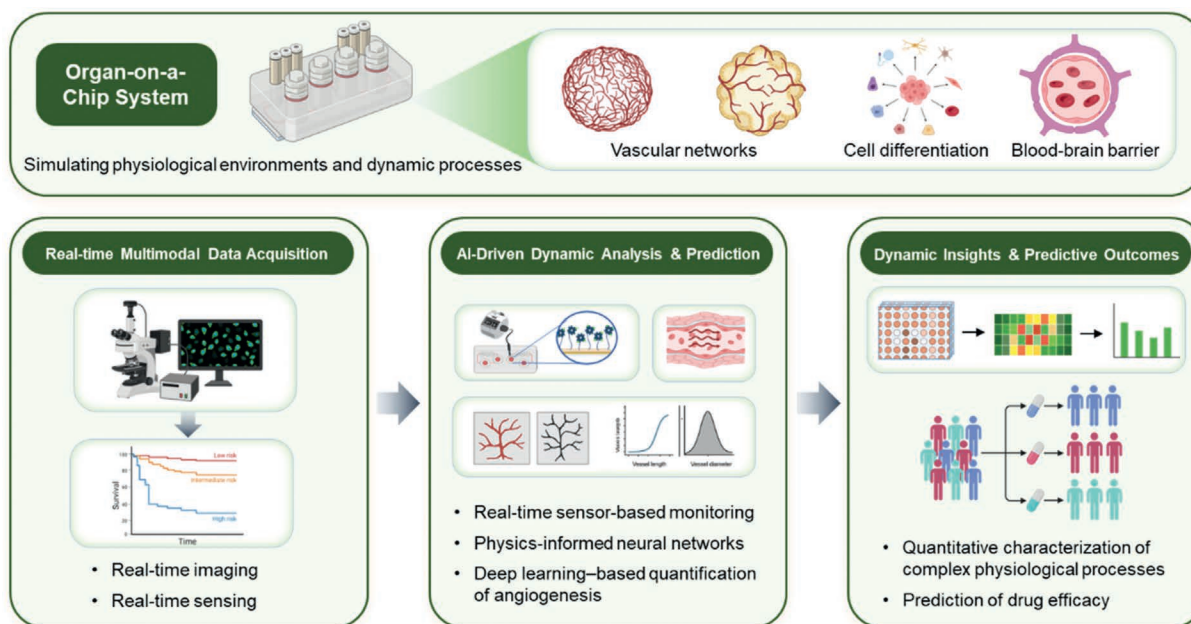


Figure 7 Schematic workflow of AI-enabled real-time monitoring and dynamic assessment in OoC HTS Systems. This figure is created with BioRender.com.

successfully forecasted cellular differentiation outcomes four weeks later. Concurrently, a MATLAB-based algorithm was deployed to monitor ciliary beat frequency and mucociliary clearance functionality in real time. This automated, image-based analytical pipeline achieved a prediction accuracy of 89%, enabling both early prognostication of differentiation status and continuous functional monitoring. This approach enabled comprehensive, multi-level, real-time assessment of cellular differentiation, ciliary function, and overall barrier integrity. By embedding AI into OoC workflows, researchers have markedly improved the fidelity, reproducibility, and throughput of chip-based models but also significantly reduced experimental costs, thereby offering a robust and scalable framework for respiratory drug screening and physiological assessment.

Cancer metastasis potential prediction. Aiming at predicting the brain metastasis potential of cancer cells, Oliver et al.¹⁹³ integrated advanced live-cell imaging with AI. By monitoring cancer cell behaviors within a microfluidic blood–brain barrier-on-a-chip system (μ BBN), they captured high-resolution, 3D datasets *via* confocal microscopy, detailing cellular volume, spatial position, and morphological parameters. Custom software was used to extract dynamic behavioral features, including volume extravasation percentage, cell-to-endothelium distance, and sphericity, which were then used to train ML models such as neural networks and random forests. The AI models demonstrated excellent predictive performance (AUC = 0.95, PPV = 0.91, NPV = 0.85) and maintained high accuracy across patient-derived xenograft (PDX) samples (AUC = 0.97). This integrated platform not only enables precise identification of metastatic-prone cancer subclones but also offers a powerful tool for advancing personalized diagnostics and targeted therapeutics.

Training-free video super-resolution. Building upon previous efforts, recent advances in unsupervised learning and physics-informed modeling have successfully mitigated persistent issues in supervised frameworks, namely the high cost of data labeling and poor generalizability under distributional shifts. These emerging paradigms offer robust, label-free solutions for modeling complex biological dynamics in OoC systems. In this vein, Cascarano et al.¹⁷⁶ introduced the Recursive Deep Prior Video (RDPV) algorithm (Fig. 6E), a training-free super-resolution approach for enhancing temporal microscopy videos in OoC applications. Built upon the Deep Image Prior (DIP) architecture, RDPV incorporates recursive update rules and total variation regularization to enable high-fidelity reconstruction of video frames without the need for pretraining. The resulting high-resolution videos significantly improved cell detection rates (from 51% to 100%), minimized trajectory switching errors, and enabled more accurate tracking of cell motility and interactions, which are key biomechanical parameters in drug screening studies. Moreover, RDPV demonstrated strong robustness to noise and variability across experimental settings, making it broadly applicable to diverse OoC contexts. Compared to conventional supervised approaches, RDPV eliminates the need for extensive labeled datasets, reduces computational burden, and supports real-time implementation. It thus represents a powerful tool for improving the resolution, reliability, and biological insight of dynamic cell behavior analysis in OoC drug screening workflows.

Physics-informed fluid inference. Cai et al.¹⁷⁷ introduced an AI-augmented velocimetry (AIV) framework that leverages physics-informed neural networks (PINNs) to integrate imaging data with fundamental fluid dynamics (Fig. 6F). In a microfluidic chip mimicking a saccular microaneurysm, AIV inferred key

hemodynamic parameters, such as 3D velocity, pressure, and shear stress fields, directly from 2D image sequences captured during flow experiments. These outputs demonstrated strong concordance with traditional computational fluid dynamics simulations. However, unlike conventional 3D reconstruction methods that require precise inlet and outlet boundary conditions, the AIV model can learn flow fields directly from imaging data, without prior knowledge of flow constraints. This capability is particularly critical for modeling complex biological fluids such as blood and for elucidating hemodynamics in pathophysiological microvascular environments like aneurysms.

The aforementioned studies have demonstrated the powerful synergy between AI and OoC technologies in biomedical research. Intelligent monitoring systems built on deep reinforcement learning algorithms, along with multimodal microscopic imaging techniques and high-throughput data processors, now enable real-time capture and precise analysis of dynamic cellular behaviors during drug screening on organ chips. These systems facilitate continuous tracking of complex biological events that unfold across space and time, including morphological changes, collective migration patterns, intercellular signaling networks, and metabolite exchanges. Together, they support the construction of 3D + dynamic databases with subcellular spatiotemporal resolution. Such platforms provide high-dimensional, traceable, and physiologically relevant experimental datasets that enable the quantitative evaluation of cell–cell and cell–matrix interactions under pharmacological perturbations. This, in turn, significantly enhances the predictive power for drug toxicity, target validation, and mechanistic pharmacodynamics.

6. Conclusions and perspectives

The synergistic integration of AI with microfluidic technologies is redefining the landscape in drug screening. This review comprehensively illuminates its impact across a multidimensional spectrum, from single-cell analysis (1D), multicellular arrays (2D), and 3D spheroids to sophisticated OoC systems (3D+), profoundly elevating screening throughput, accuracy, and biological relevance. Specifically, at single-cell level (1D), AI has enabled microfluidics to precisely dissect cellular heterogeneity and facilitate high-throughput sorting, significantly enhancing sensitivity and predictive accuracy in drug response assessment. For multicellular models (2D), AI, particularly through ML and CNNs, has catalyzed scalable, label-free, and multiparametric interrogation of complex cellular behaviors, substantially streamlining drug toxicity and efficacy evaluation. In 3D spheroid drug screening, AI has orchestrated a revolution in spheroid fabrication optimization, quality control, and quantitative assessment, pivoting towards label-free viability estimation and real-time immune cell tracking, thereby fortifying physiological fidelity. For OoC systems, AI has unveiled unprecedented capabilities for comprehensive multi-modal and multi-omics data analysis, seamlessly coupled with real-time dynamic monitoring, yielding profound insights into complex drug action mechanisms and accelerating the trajectory towards precision medicine. Moreover, advances in AI-integrated microfluidic platforms for biosensing have also accelerated progress in wearable technologies, thereby extending the scope of microfluidic drug screening beyond preclinical discovery into patient-tailored clinical decision-making^{194,195}.

Despite these profound advancements, critical constraints persist, hindering the widespread translation of AI-microfluidics from promising prototypes to routine practice in drug screening.

Realizing the full spectrum of its potential demands concerted, multidimensional efforts across several pivotal domains:

Hardware advancements and standardization. Current microfluidic platforms, while sophisticated, frequently encounter limitations in throughput for ultra-large-scale compound libraries, full automation, cost-effectiveness, and inter-experimental consistency. Specifically, the seamless integration of diverse hardware components (e.g., pumps, sensors, actuators) with real-time AI analytics often presents significant engineering and communication bottlenecks, impacting overall system robustness and scalability. Future endeavors must prioritize enhancing overall system automation and analytical throughput, alongside a substantial reduction in per-assay costs. This necessitates the development of modular, reconfigurable microfluidic architectures that seamlessly integrate diverse sensing modalities¹⁹⁶. The integration of edge AI capabilities directly into microfluidic devices will be pivotal for enabling real-time, on-chip data processing and autonomous decision-making, thereby minimizing latency and enhancing system responsiveness for complex experimental control. Crucially, rigorous standardization of chip designs and fabrication processes, particularly for organoids and OoC systems, is imperative to ensure robust reproducibility and cross-laboratory comparability, thereby fostering wider adoption and accessibility to these powerful platforms^{197,198,153}. Furthermore, community-driven open-source platforms such as Metafluidics¹⁹⁹, by facilitating the sharing of standardized microfluidic device designs and assembly protocols, underscore the potential for improved chip operability and experimental comparability, thus fostering broader data interoperability. To further enhance utility, developing standardized image annotation protocols for AI model training is essential, providing practical guidance for consistent data labeling and ensuring standardized datasets across various microfluidic applications.

Software innovation and algorithmic robustness. The prevalent reliance on “black-box” DL models fundamentally limits their interpretability, posing a significant practical hurdle for elucidating causal drug-action mechanisms and gaining regulatory acceptance. Moreover, ensuring the generalizability of AI models across heterogeneous microfluidic datasets, often characterized by inherent noise and batch effects, remains a formidable challenge²⁰⁰. Addressing this, future research should focus on advancing XAI frameworks and PINNs to enhance model transparency and predictive fidelity, aiming for AI-derived insights that are both actionable and trustworthy. Concurrently, the development of new, more robust algorithms with enhanced learning capabilities will be crucial for microfluidics-based drug screening²⁰¹. These algorithms should be adept at multimodal data fusion, transfer learning (to mitigate data scarcity), and active learning (to alleviate annotation burdens), thereby proving more economical and efficient while better suiting the specific needs of microfluidic chip-based drug screening. Furthermore, the strategic application of reinforcement learning algorithms for dynamic fluidic control will enable autonomous optimization of experimental parameters in closed-loop systems, maximizing physiological relevance and screening efficiency. Fostering open-source AI tools and platforms will be instrumental in democratizing access to these advanced analytical capabilities, enabling broader participation and catalyzing innovation. The exploratory potential of generative AI for *de novo* drug design or simulating intricate biological interactions within OoC digital twins also holds promise to significantly reshape early-stage pharmaceutical discovery^{202–204}.

Data management and interoperability. Microfluidic systems generate high-dimensional, multimodal spatiotemporal datasets,

yet often contend with limited sample sizes and heterogeneous data formats, creating a pervasive “data paradox”. Addressing this necessitates pioneering innovative data strategies, such as self-supervised learning and advanced multimodal data fusion, to overcome annotation complexities. Establishing standardized data repositories coupled with common ontologies is paramount to foster seamless data sharing and interoperability. For instance, platforms such as EveAnalytics, formerly Microphysiology Systems Database (MPS-Db)²⁰⁵, exemplify dedicated efforts to aggregate and standardize diverse microphysiological system data, thereby facilitating cross-laboratory reproducibility and comparison. Beyond centralized repositories, the adoption of universal data annotation frameworks, such as FAIR principles (Findable, Accessible, Interoperable, Reusable)²⁰⁶ and ISA-Tab format²⁰⁷, significantly enhances data compatibility and readability through structured capture of comprehensive experimental metadata across various omics and assay types. Moreover, building secure and scalable cloud-based infrastructures for data storage and analysis will be crucial to ensure data integrity, accessibility, and robust privacy protection²⁰⁸. Collectively, these initiatives are instrumental in addressing the “data paradox” by transforming heterogeneous datasets into actionable, AI-ready resources, which in turn contributes to accelerating drug candidate development and validation.

Emerging AI architectures. Transformer-based models offer exciting potential for advancing microfluidic drug screening, especially in systems involving complex and multimodal data streams. Unlike CNNs and RNNs typically limited to local feature extraction or sequential modeling, transformers offer a unified attention-driven framework capable of capturing complex, long-range dependencies across heterogeneous data types²⁰⁹. Recent advances such as Dynaformer, DRPreter, SIC50 and GraphormerDTI have demonstrated the ability of Transformers to integrate molecular, genomic, and imaging modalities for biological prediction tasks with superior accuracy and interpretability^{210–215}. Moreover, attention-based models have been successfully applied in droplet microfluidics for real-time image-based detection and sorting²¹⁶, suggesting strong potential for future adoption in microfluidic drug screening contexts. As the increasing complexity of OoC technologies and multicellular systems, emerging artificial intelligence models may offer greater scalability, enhanced flexibility, and more biologically informed decision-making compared to traditional deep learning approaches.

Regulatory and ethical frameworks. The burgeoning emergence of advanced *in vitro* models, particularly stem cell-based and brain-mimicking OoC systems, inherently introduces complex regulatory and ethical considerations²¹⁷. Proactive collaboration with key regulatory agencies (e.g., FDA, EMA) is imperative to establish agile and adaptive approval pathways that can rigorously yet flexibly evaluate AI-enhanced OoC innovations, thereby fostering responsible translation. Concurrently, addressing profound ethical concerns related to the use of human-derived cells, the potential for sentience or consciousness in complex neural OoC, and robust data privacy protocols will be paramount to ensure responsible innovation and broad societal acceptance.

In summary, realizing the full potential of AI-enhanced microfluidics hinges upon concerted, cross-disciplinary efforts, spanning bioengineering, machine learning, pharmacology, and materials science, alongside proactive engagement with regulatory bodies. By collectively addressing the aforementioned intertwined challenges through coordinated technological innovation and community consensus, these intelligent platforms are poised to

fundamentally transform drug screening. This progress will enable the development of more precise, scalable, and cost-effective assays that accurately capture biological complexity from single cells to whole organs, thereby directly accelerating personalized therapeutic development and significantly reducing reliance on animal models.

Acknowledgments

We gratefully acknowledge the financial supports from Non-communicable Chronic Diseases-National Science and Technology Major Project (2024ZD0528200, China), Hubei Provincial Natural Science Foundation of China (2025AFB210, 2023AFA052), and National Natural Science Foundation of China (22574043).

Author contributions

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Conflicts of interest

The authors declare no conflict of interest.

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