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REVIEW

Artificial intelligence-enabled organoids and organ-on-a-chip for pioneering biomedical platforms

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Abstract

Organoids and organ-on-a-chip (OoC) are two innovations used in biomedical applications for drug evaluation, disease modeling, and precision medicine. These microphysiological systems (MPSs), including integrated OoC, mimic the physiological or pathological conditions in human tissues or organs, overcoming the limitations of conventional cell culture and animal model experiments. The integration of artificial intelligence (AI) with MPS has further enhanced its capabilities, enabling advanced data processing, real-time monitoring, and predictive modeling. This review highlights the applications of AI-enabled organoids and OoC systems in MPS biomedical research, namely improving biomaterial development, spatial and functional optimization, growth factor combinations, and fine-tuning of microenvironmental stimuli. AI can also automate data analysis and enable real-time insights into cellular behavior in OoCs. Finally, we address the challenges and limitations associated with these MPS technologies and highlight future directions for their integration with AI.

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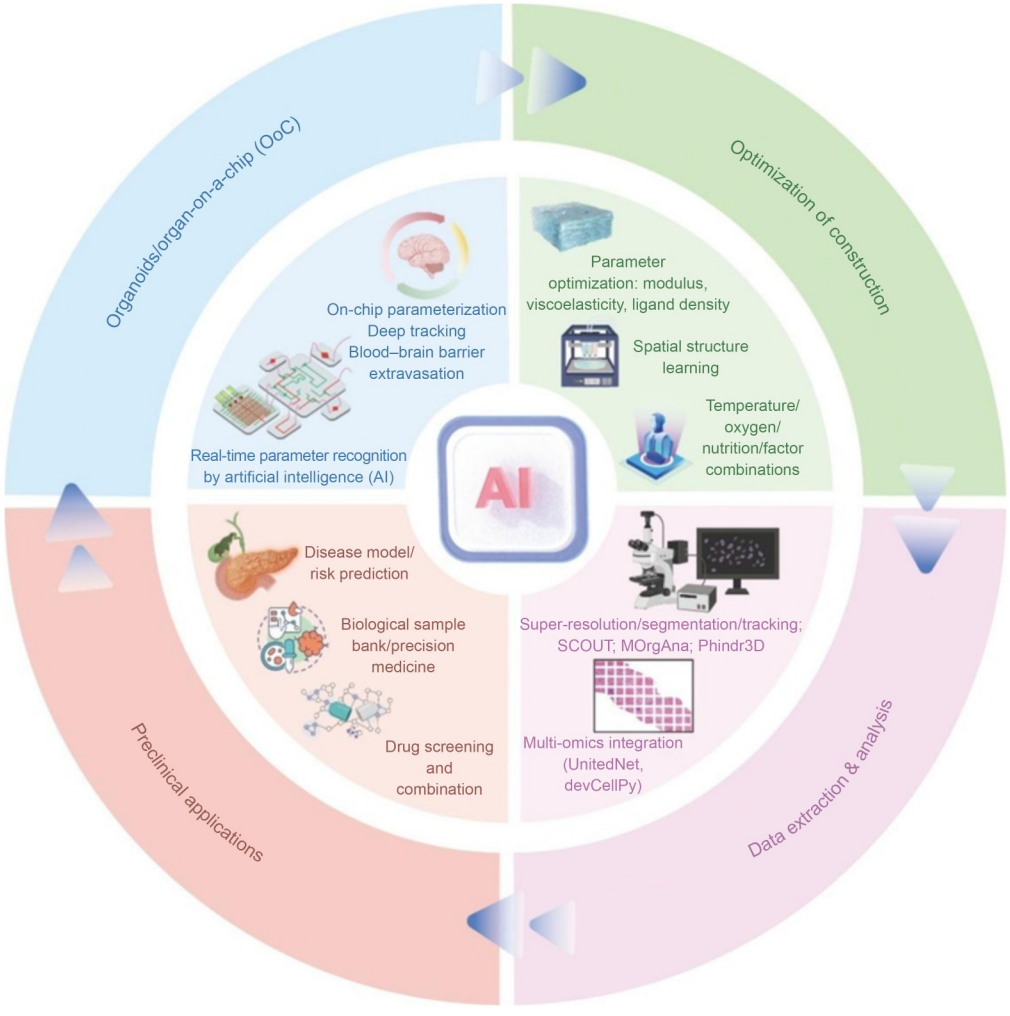
Graphical abstract



Keywords Artificial intelligence · Organoids · Organ-on-a-chip · Organoids-on-a-chip · Microphysiological systems

1 Introduction

In recent years, organoids and organ-on-a-chip (OoC) systems have rapidly emerged as innovative tools for drug discovery, disease modeling, and personalized medicine [1]. Organoids are stem cell-derived three-dimensional (3D) organizational analogs that mimic the cellular diversity, architectural complexity, and functional attributes of native organs or tissues [2]. OoC technology adds living cells to microfluidic devices to emulate dynamic physiological processes, such as fluid flow and shear stress, thereby enabling the recapitulation of organ-level functionality or multiorgan interactions [3]. This OoC platform combines the 3D complexity and cellular fidelity of organoids with the dynamic and physiological relevance of microfluidic OoC systems [4]. However, multi-OoC or multi-organoids-on-a-chip enhances the fidelity of in vitro models, replicating the interactions among multiple organs and systemic influences within the human body and offering a biomimetic microenvironment to develop a more comprehensive human-on-a-chip model [5]. Similarly, microphysiological systems (MPSs) mimic the physiological or pathological conditions of human tissues or organs, overcoming the limitations of two-dimensional (2D) cell cultures and animal experiments [6].

Conventional organoid/OoC analysis depends largely on manual annotation, conventional image processing algorithms, and low-throughput experimental workflows, which are inevitably limited by subjective human judgment, labor-intensive operations, and poor adaptability to complex biological variability [7]. For example, organoid morphology

or OoC-derived physiological signals often require hours of expert labor for each sample and suffer from inter-observer variability when manual quantification and analysis are performed, whereas conventional computational methods may not capture subtle, nonlinear biological patterns in heterogeneous datasets [8].

The rise of artificial intelligence (AI) has amplified the potential of integrated MPS platforms, owing to the rapid development of data-driven biology [9]. AI technologies, including machine learning, deep learning, and big data analytics, have become indispensable tools for optimizing and interpreting complex biological systems [10]. AI has enhanced MPS research through advanced machine learning models, such as convolutional neural networks (CNNs) and generative algorithms, which can optimize cell culture protocols and experimental designs as well as analyze high-dimensional datasets with unparalleled speed and accuracy [11]. AI offers transformative tools for improving biomaterial development, spatial and functional optimization, growth factor combinations, and fine-tuning of microenvironmental stimuli to benefit organoid-related research and development [12]. Concurrently, the integration of AI into these platforms has revolutionized data harvesting, analysis, and optimization, offering greater precision and scalability in preclinical and biomedical studies [13].

AI is also indispensable for automating data analysis and enabling real-time monitoring of cellular behaviors in organoids and OoCs [14]. AI can be used to analyze high-throughput data from imaging, transcriptomics, and proteomics to refine culture conditions, predict cellular responses, and identify novel therapeutic targets on MPS platforms [15]. For example, machine learning algorithms can process time-lapse images to monitor organoid growth or subtle morphological changes in real time. Conversely, predictive models can simulate drug pharmacokinetics in microfluidic systems [16].

This highlights the innovativeness of AI-enabled organoid/OoC platforms in terms of efficiency, accuracy, and scalability compared to conventional methods [17]. AI techniques, particularly deep learning models, enable end-to-end automation of data processing, reduce the analysis time from hours to minutes per sample, and minimize human bias [18]. This is achieved by leveraging large-scale training datasets to learn complex biological correlations by capturing high-dimensional, nonlinear features that conventional approaches overlook for enhanced accuracy and predictive performance, as shown in organoid maturation status classification and OoC toxicity prediction [19]. Scalability is another pivotal advancement; unlike conventional methods that are limited by sequential processing and limited data integration capabilities, AI-driven pipelines can seamlessly integrate multimodal data (imaging, transcriptomics, or electrophysiology) from high-throughput organoid/OoC platforms, and simultaneously process thousands of samples without compromising performance [20]. Taken together, these technological breakthroughs establish AI as a transformative tool that overcomes the core limitations of conventional approaches, enabling precise, efficient, and scalable characterization of organoids and OoCs for applications in translational studies [21].

This review explores the cutting-edge applications of AI-assisted organoids and OoC technologies in biomedical research (Fig. 1). This AI-enabled integrated platform advances experimental workflows as well as the reproducibility and scalability of MPS models [22]. Similarly, intelligent OoC (iOoC) systems integrated with real-time monitoring sensors enable dynamic feedback loops, enhancing the precision and reproducibility of the experimental outcomes [23]. The integration of MPS technologies with rapidly advancing AI is accelerating our understanding of human biology to provide more effective and personalized therapeutic strategies [24]. The integration of AI with MPS has further bolstered its capabilities, enabling advanced data processing, real-time monitoring, and predictive modeling [25]. We address the challenges and limitations of these MPS technologies while highlighting future directions for their integration with AI and with emerging biomedical platforms.

2 Overview of AI and integration with biology

AI is a subdiscipline of computer science that seeks to emulate human cognitive abilities such as problem-solving, learning, and decision-making. It has become an interdisciplinary domain that integrates fields such as mathematics, neuroscience, and psychology to strengthen its capabilities [26]. The term "artificial intelligence" was formalized during the Dartmouth Conference in 1956 by John McCarthy, a pivotal event that laid the foundation for AI as an academic field. The initial era (1950s–1970s) of AI development focused on symbolic reasoning and expert systems that relied heavily on rule-based approaches [27]. AI witnessed a resurgence in the 1980s with the evolution of machine learning algorithms, and in the 1990s and early 2000s, with the rapid growth of high-performance computing and the availability of data on the Internet [28].

Modern AI technologies rely on machine learning, particularly deep learning, which uses multi-layered neural networks to extract patterns and features from raw data. For instance, breakthroughs such as AlphaGo and AlphaFold have revealed AI's ability to solve problems that were once considered too complex for machines. Despite its initial theoretical origins, AI has driven technological innovations in robotics, healthcare, genomics, drug discovery, and natural language processing. Its applications in domains such as

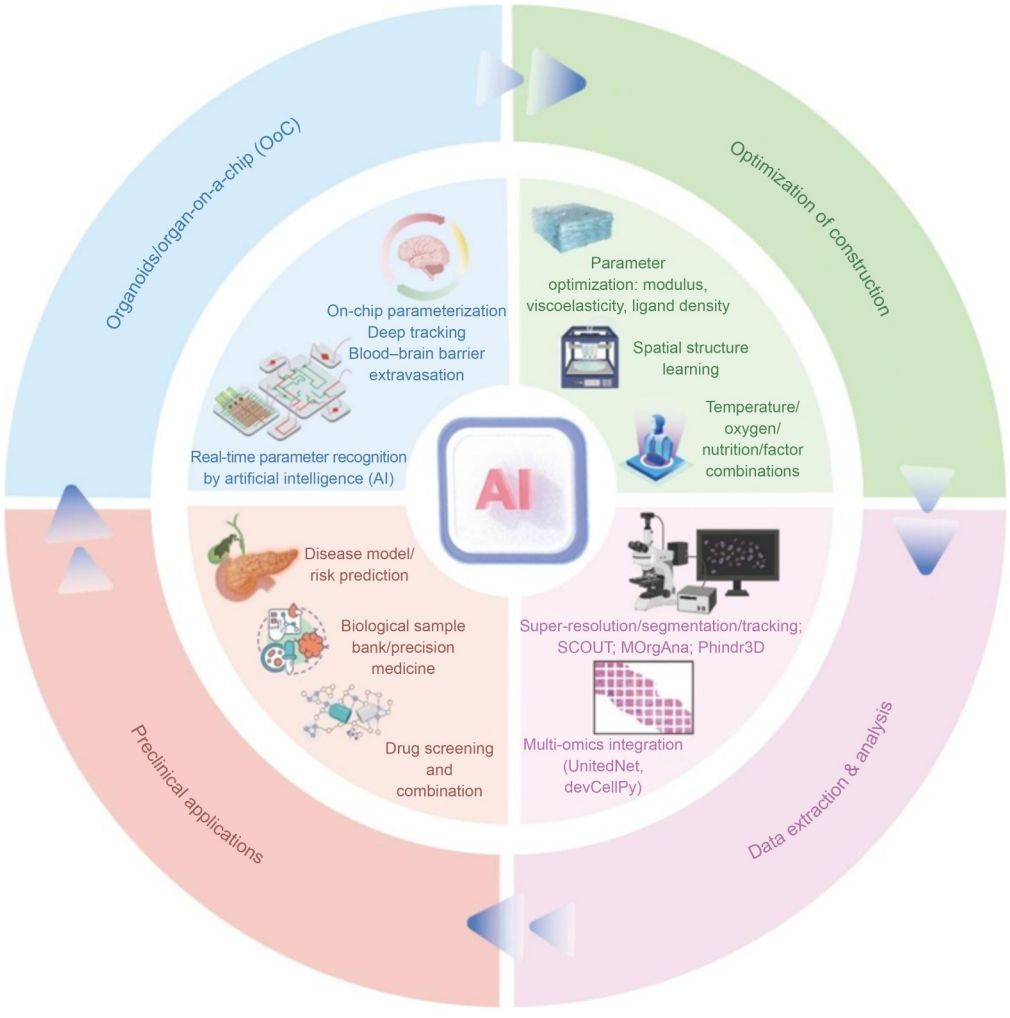


Fig. 1 Schematic of artificial intelligence (AI)-enabled organoids and organ-on-a-chip (OoC) for optimization of construction, data extraction, and analysis, and preclinical applications, as well as the specific presentation and application methods for pioneering biomedical platforms

organoid/OoC fabrication and biomedical data analysis are a key example of the integration of biology and AI [26, 27].

2.1 Types of machine learning

Machine learning, a subdomain of AI, is the backbone of intelligent system development. It provides algorithms that learn iteratively from data to adapt to new environments. Machine learning is primarily classified into three categories: supervised, unsupervised, and reinforcement [26] (Fig. 2a). Supervised learning involves training models on labeled datasets to predict outcomes. Standard methodologies include decision trees, support vector machines (SVMs), and neural networks, which have proven to be highly effective for tasks such as disease prediction and patient risk stratification [29]. Conversely, unsupervised learning leverages unlabeled data to identify underlying patterns, as exemplified by clustering methods such as *k*-means or principal component analysis, which are often performed to reduce dimensions and detect patterns in high-dimensional genomic or telecom datasets [10, 30]. Reinforcement learning allows AI systems to optimize behavior through feedback mechanisms, particularly in dynamic environments such as robotics and control systems. These machine learning techniques collectively contribute to the scalability and precision of AI applications across various biomedical and industrial domains by extracting features from complex datasets such as those generated in genomics research or organoid imaging [10].

2.2 Models of machine learning

Different machine learning models have been used based on the specific nature of the tasks and the characteristics of the data [31] (Fig. 2b). CNNs, for instance, excel at image-based tasks, including tumor classification in medical imaging and organoid structural analysis, given their ability to learn spatial hierarchies. CNN-based models have been

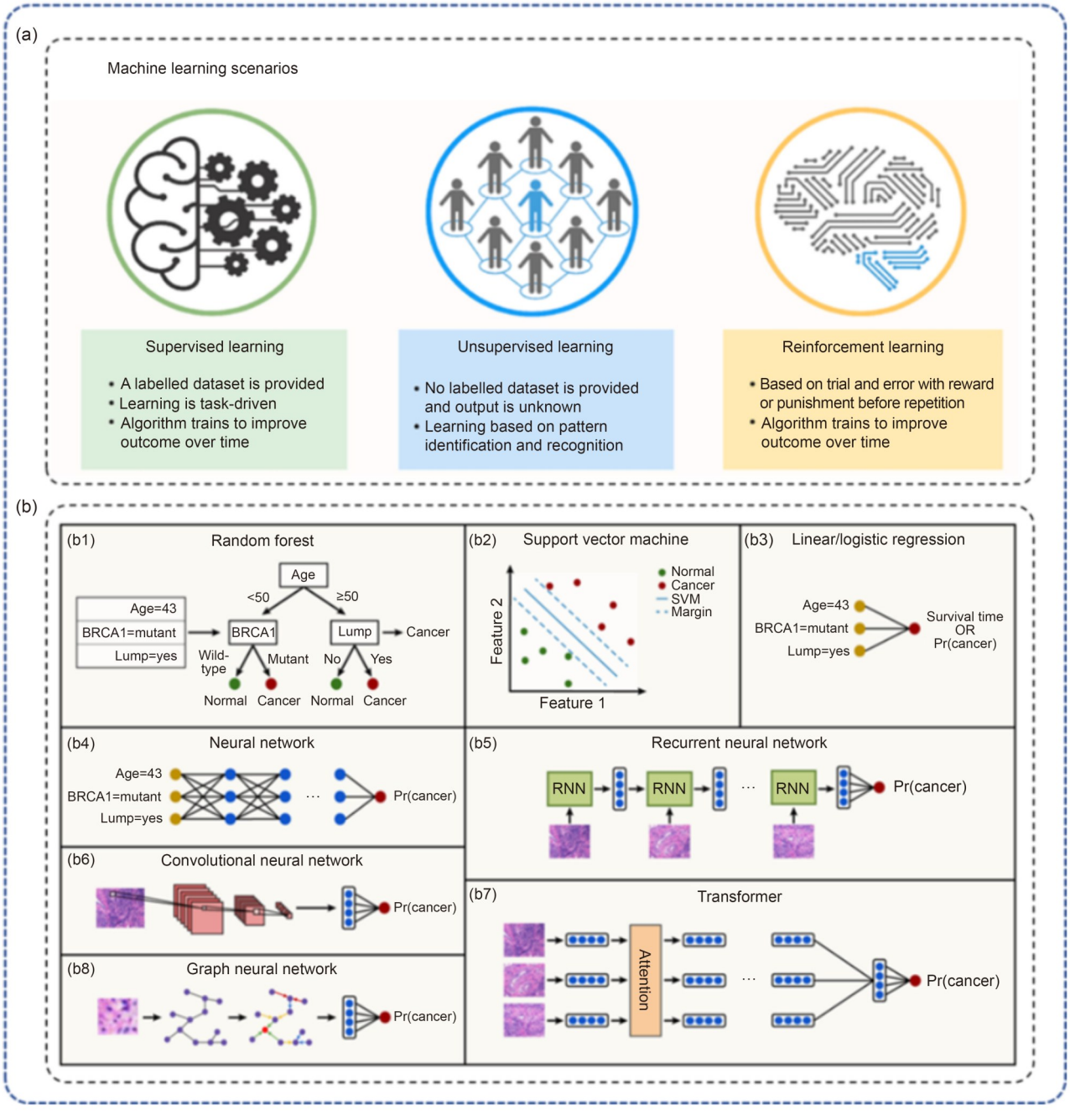


Fig. 2 A brief introduction to machine learning, a cutting-edge technology derived from the development of artificial intelligence (AI). (a) The main types of machine learning: supervised learning, unsupervised learning, and reinforcement learning. Reproduced from [26], licensed under CC BY-NC-ND. (b) Common machine learning models, such as random forest (b1), support vector machine (b2), linear/logistic regression (b3), neural network (b4), recurrent neural network (b5), convolutional neural network (b6), transformer (b7), and graph neural network (b8). Reproduced from [31], with permission from Elsevier Inc.

successfully applied to breast cancer diagnosis, significantly improving sensitivity and classification accuracy over conventional softmax classifiers [32]. Recurrent neural networks (RNNs) are instrumental for sequence modeling tasks, such as natural language processing, and for healthcare applications, such as medical transcript generation [33]. Similarly, SVMs efficiently handle high-dimensional data and are therefore widely used in genomics for tasks such as differential gene expression classification [34]. Hybridized models, such as a combination of CNN and SVM, have also emerged to capitalize on the strengths of each algorithm; for example, CNNs extract image features, which are then classified by SVMs to improve diagnostic outcomes [32].

Other innovative architectures, such as graph neural networks, can be used to analyze complex network-based data in molecular biology, helping discover new drugs and predict biomolecular interactions [35]. Similarly, advances in deep learning models, such as recurrent CNNs, are especially adaptable due to incorporating gated mechanisms for biological plausibility, outperforming conventional models in object recognition tasks [36]. These models underscore the versatility of machine learning techniques for overcoming diverse data-driven challenges across domains, particularly in biomedical research, drug screening, and personalized medicine.

3 AI-driven optimization of organoids

Organoids are self-organized 3D in vitro systems that mimic the functionality of native organs. These complex constructs require comprehensive optimization of chemical, biological, and physical parameters [37]. Conventional techniques for constructing organoids rely heavily on manual experimentation and trial-and-error. However, AI, particularly machine learning, has emerged as a paradigm-shifting technology thanks to its ability to process large-scale datasets, discover patterns, and make accurate predictions, expediting organoid-related research and development [38]. These features are improving biomaterial development through optimization of spatial and functional components, growth factor combinations, and microenvironmental stimuli [39]. Thus, AI-driven optimization of organoids has broad implications for their applications in personalized medicine, high-throughput screening, and disease modeling.

3.1 AI-driven optimization of organoid construction strategies

AI can help design and optimize biological materials that serve as effective base materials (such as matrix gels) for organoid construction. Widely used natural matrices such as Matrigel pose challenges such as batch-to-batch variability, uncontrollable microenvironments, and immunogenicity risks [40]. In this context, synthetic and semisynthetic hydrogels have garnered attention for their tunable properties and biocompatibility [41]. However, their development remains complex and challenging to achieve through supervision and verification owing to the inherently multidimensional relationships between chemical properties, structural configurations, and biological behaviors.

AI has advanced biomaterial design. Verheyen et al. [42] relied on machine learning models at each stage to develop granular alginate hydrogels optimized for injectability, rheological properties, and cellular interactions. Similarly, Li et al. [43] used machine learning to attribute the chemical features of over 2000 peptides to their ability to self-assemble into hydrogels, facilitating the efficient design of peptide hydrogels with predefined stiffness and uniform nanostructures. Furthermore, Suwardi et al. [44] highlighted the broader applications of AI in expediting biomaterial evolution, in which high-throughput experiments and simulations, combined with machine learning, shifted biomaterial discovery from a trial-and-error model to a data-driven paradigm.

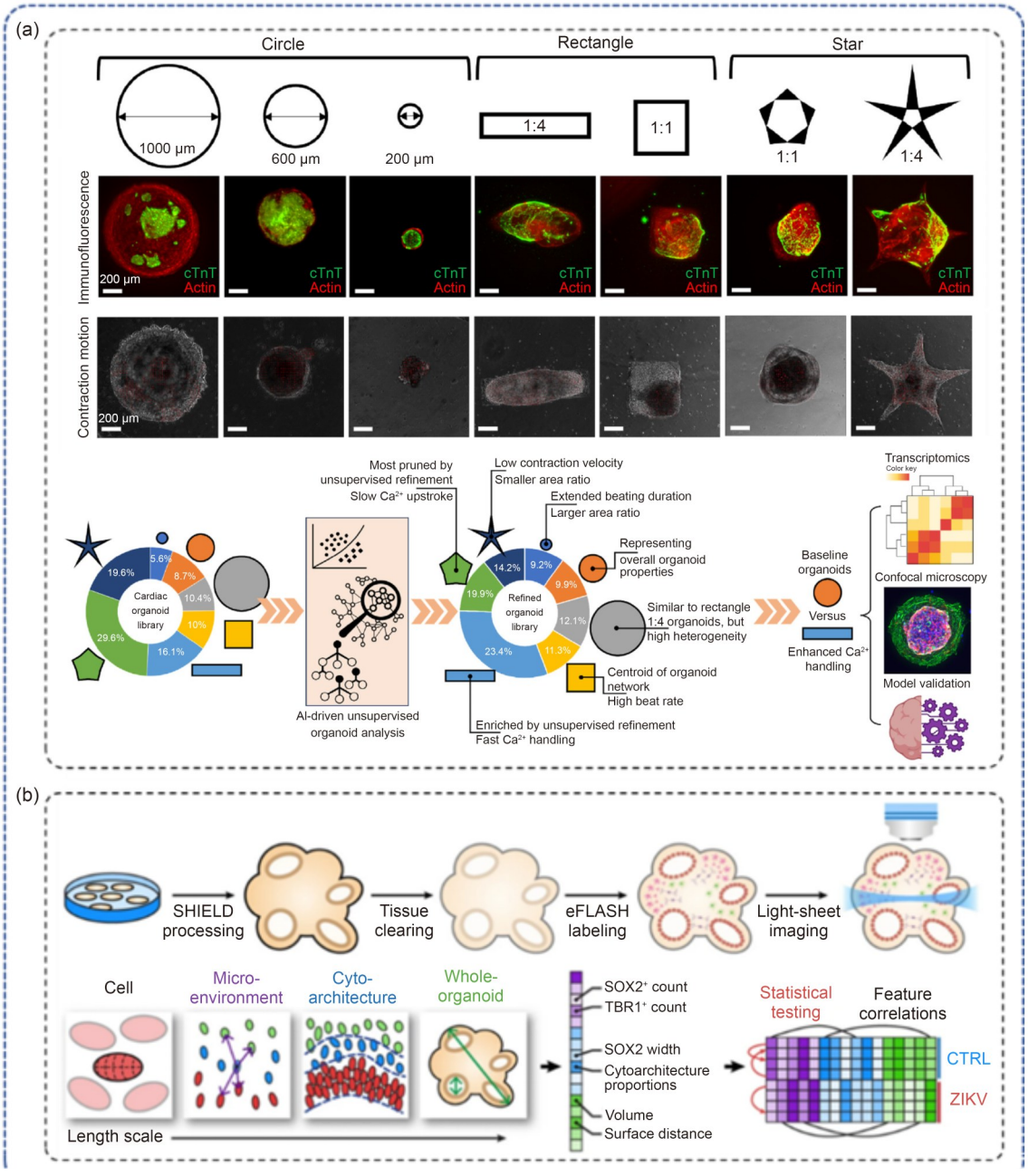
Machine learning has also been used to assess and optimize the spatial structure of synthetic hydrogels. Using a single method, Liaw et al. [45] assessed various hydrogel configurations, including fibrous scaffolds and 3D bioprinted constructs, based on their ability to replicate the microenvironments of the liver and cancer models. As data-driven designs have been shown to mimic the complexity of native tissue, the predefined hydrogels were then refined using AI-driven methodologies. Similarly, Gjorevski et al. [46] designed fully synthetic matrices for intestinal organoid cultures and fine-tuned extracellular matrix parameters, including stiffness and biochemical composition. This approach identifies distinct microenvironmental requirements for stem cell proliferation versus differentiation, thereby outperforming conventional animal-derived matrices such as Matrigel.

AI has guided the optimization of the spatial structure of matrix gels, which is critical for sustaining cellular behaviors such as migration, proliferation, and differentiation [47]. Computational tools integrated with imaging technologies have emerged as vital enablers of spatial optimization. For example, Hasib et al. [48] used CNNs to analyze electrophysiological signals in human embryonic stem cell (hESC)-derived brain organoids, offering nuanced insights into structural and functional development. Similarly, Kowalczewski et al. [49] combined unsupervised machine learning and micropatterning techniques to create a library of cardiac organoid geometries and identify unique structure-function correlations that enhanced organoid performance when clustered by functional similarity. AI-driven analytical approaches were applied to elucidate the functional heterogeneity of cardiac organoids and to provide mechanistic insights into the physiological enhancement by a cardiac organoid library with seven geometric designs. This integration of organoid engineering and machine learning dissects the unique functionalities of geometric designs and paves the way for more controlled and optimized organoid design (Fig. 3a).

Achieving consistent results using conventional manual protocols remains highly variable and inefficient. To combat this, AI provides automated cell culture protocols to standardize the intricacies of cell culture conditions, including temperature, nutrients, oxygen levels, and growth factor composition, which are foundational for organoid

development. To exemplify this, Kanda et al. [50] introduced a robotic AI platform that integrates Bayesian optimization and explores 143 stem cell culture conditions systematically. In this system, retinal pigment epithelial cell production increased by 88% compared with pre-optimized cultures, a significant advancement in reproducibility and efficiency.

AI expedites the identification and combination of appropriate growth and inducing factors, such as Wnt, bone morphogenetic protein (BMP), and transforming growth factor-β (TGF-β), for proper organoid maturation. Singaraju et al. [51] developed an "Organalysis" application that trains machine learning models to identify key growth factors for cardiac organoid differentiation. This tool not only automates the preprocessing of data from imaging, but also performs lasso-regularized regression analyses to predict optimizing factors. Likewise, Zhu et al. [52] demonstrated the ability of deep neural networks (DNNs) to recognize early neural stem cell (NSC) differentiation patterns without labeled datasets, emphasizing the generalizability and accuracy of AI-driven predictive models for cell culture optimization. Granular screening platforms have also been instrumental. Notably, Ao et al. [53] harnessed a high-throughput AI-guided microfluidics system to dynamically track T-cell and tumor organoid interactions, thereby identifying key epigenetic modulators of drug resistance in combination therapies.



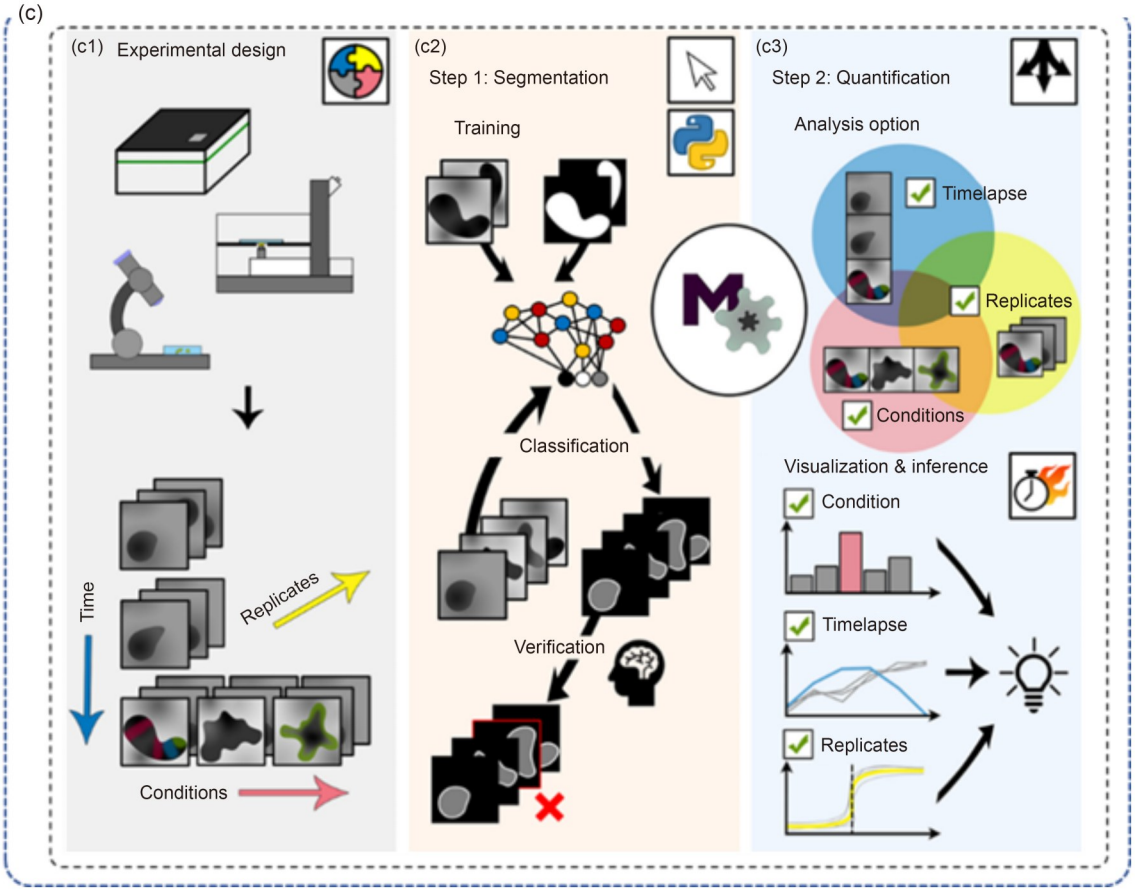


Fig. 3 Machine-learning-guided image analysis of organoid scale. Specific studies of artificial intelligence (AI) in the data of organoids are shown as follows. (a) Summary of AI-driven unsupervised organoid analysis and representative fluorescence images of cardiac organoids stained in seven geometric designs. Reproduced from [49], licensed under CC BY-NC-ND. (b) Pipeline for multiscale hyperdimensional analysis of organoids via single-cell and cytoarchitecture analysis of organoids using unbiased techniques (SCOUT). Reproduced from [58], licensed under CC BY 4.0. (c) MOrgAna workflow schematic. Reproduced from [15], licensed under CC BY 4.0

AI-enabled tools have played a crucial role in optimizing external stimuli and environmental regulation parameters that influence organoid development, especially physical cues such as light, mechanical stimuli, and electrical fields. So et al. [54] developed deep-learning-driven epidermal piezoresistive sensors for the precise quantification of mechanical stimuli, significantly improving the accuracy of pressure and deformation mapping in engineered tissues. Hasib et al. [48] also used CNNs to extract functional readouts from brain organoids based on electrophysiological recordings, fostering the classification of organoid conditions and mutations.

Ultimately, AI-driven methodologies have deepened our understanding of and accelerated the optimization of organoid construction. AI has reshaped the trajectory of organoid research from biomaterial design and spatial structure discernment to automated cell culture protocols and regulation of environmental cues. Machine learning can handle multidimensional datasets and high-throughput experimentation, realizing novel opportunities for personalized medicine, disease modeling, and drug screening by seamlessly complementing conventional approaches.

3.2 AI-accelerated data extraction and analysis of organoids

Accurate and efficient image data analyses are the basis of the functional assessment of organoids by bridging technological advancements and biological insights. The integration of AI and machine learning in organoid research has significantly improved the speed, accuracy, and scalability of data extraction, enabling deeper analyses of multiscale and multi-modal datasets [55]. This subsection outlines progress in AI-enabled organoid data analysis across distinct dimensions, including morphology, cellular dynamics, organoid-scale development, tissue organization, and multiomics.

AI-enabled analysis of organoid morphology from fluorescence microscopy datasets and time-lapse images is becoming increasingly indispensable for organoid analysis. Qiao et al. [16] introduced a novel deformable phase-space

alignment time-lapse image super-resolution (DPA-TISR) neural network, which significantly enhanced organoid image analysis through superior temporal consistency and fidelity. In this study, a DPA-TISR was developed by leveraging the large-scale fluorescence microscopy dataset BioTISR, which contains time-lapse images of biological specimens, including organoids. This model decouples the propagation of temporal information and neighbor feature alignment and then optimizes these components to outperform existing single-image super-resolution (SISR) and TISR methods. DPA-TISR adaptively refines cross-frame alignment in the phase domain, achieving high-fidelity SR reconstructions across more than 10,000 time points of multicolor live-cell imaging and capturing intricate organoid dynamics, such as mitochondrial and cytoskeletal interactions.

Additionally, the authors advanced DPA-TISR into a Bayesian framework by incorporating a confidence calibration mechanism to quantify inference uncertainty, improving the reliability of organoid analyses. By combining an expected calibration error minimization strategy with a combined loss function, Bayesian DPA-TISR reduces overconfidence, enabling error-aware SR imaging within an efficient 30–50-min refinement process. This is essential for long-term organoid observations to ensure accurate and long-term morphological and cellular assessments of organoids. The open-access BioTISR dataset complemented the previous BioSR dataset, promoting further development of TISR techniques. This study underscored the necessity of advanced image processing in organoid research; it provided a scalable, confidence-quantified tool that harmonizes AI innovations with biological insights, which is pivotal for functional and developmental studies in organoid systems.

As the foundation for organoid formation, the matrix gel is the first part of morphological analysis. Machine learning models trained on image datasets of matrix gels can quantify critical features, such as porosity and fiber alignment, to help optimize organoid culture conditions [15]. At the cellular level, morphological and behavioral changes during differentiation or in response to external interventions are key markers of organoid functionality. AI models, such as Phindr3D, enable robust, segmentation-free phenotyping of individual cells without losing heterogeneity in datasets, making them applicable for high-content image analysis of neurons or organoid morphogenesis [56]. Moreover, methods such as deep-learning-based NSC differentiation analysis have been shown to accurately predict cellular fate, underscoring the value of machine learning for cell-level insights [57].

Albanese et al. [58] developed single-cell and cytoarchitecture analysis of organoids using unbiased techniques (SCOUT), an automated pipeline for the multiscale morphological assessment of intact cerebral organoids across molecular, cellular, spatial, and system-wide scales. The platform integrates SHIELD tissue clearing, eFLASH antibody labeling, and light-sheet fluorescence microscopy to rapidly acquire high-resolution 3D datasets of whole organoids, each imaged in approximately 15 min. Subsequent automated analysis, powered by algorithmic and CNN-based methods, extracted approximately 300 features, including cell population distribution, ventricle morphology, and cytoarchitectural patterns, from datasets comprising approximately 100 million cells across 46 organoids. This pipeline was used to assess organoid development, compare culture protocols, and quantify Zika virus (ZIKV)-induced pathology, revealing reductions in organoid size, ventricle volume, and spatial reorganization of $TBR1^+$ neurons, including a newly identified sparse population in differentiation-rich regions (Fig. 3b).

SCOUT's strength lies in its ability to comprehensively quantify morphology without sacrificing the spatial context, as demonstrated by its analysis of maturation-related changes and protocol-driven variability. For instance, it measured an approximately 50% reduction in $PAX6^+$ progenitors and $TBR1^+$ cells at 4 d post-ZIKV infection, along with an approximately 33%–50% decrease in ventricular and neuronal layer thickness at 14 d; this is not only consistent with prior studies, but it also uncovered novel spatial features, such as shifts in ventricle frequency. The open-source availability of pipelines on GitHub and via Docker enhances accessibility and promotes customization for diverse research applications. By focusing on nuclear markers (SOX2 and TBR1) in this initial study, SCOUT successfully quantified 3D tissue architecture across scales, offering a robust framework for standardized, high-throughput morphological assessment of cerebral organoids and their responses to experimental perturbations.

One of the most critical challenges in organoid research is its dependence on fluorescent labeling of distinct cellular structures for dynamic analyses. Fluorescence labeling is a highly effective method for characterizing the structural and cellular features of organoids. However, it can alter the inherent cellular dynamics and the original state or structure of the organoids, as well as affect subsequent analyses. To circumvent the need for conventional labeling, AI uses automated algorithms for cell segmentation and tracking. For example, a high-throughput organoid image dataset was developed along with a DNN for organoid detection and dynamic monitoring [59]. This DNN-based approach detects and matches organoids across sequential frames of time-lapse images, enabling precise and rapid analysis of organoid growth patterns throughout culture. Similarly, OrganoidTracker includes advanced time-lapse microscopy analysis by leveraging a CNN for cell tracking, while correcting errors and bridging manual and automated methodologies [60].

AI enables automated image analysis without labeling bias in organoids. Gritti et al. [15] demonstrated this with

MOrgAna, a Python-based software designed to automate the image analysis of organoids and thereby overcome the challenge of processing large volumes of imaging data without introducing labeling biases. The tool includes two steps: initial segmentation using bright-field images, followed by the quantification of morphological and fluorescence features. Pixels are then grouped into background, organoid, and edge categories for precise boundary detection, even in complex organoid structures surrounded by debris, thanks to machine learning techniques such as logistic regression and multilayer perceptrons. This approach avoids reliance on fluorescence labeling for segmentation, which can introduce bias due to inconsistent marker expression, making it particularly suitable for patient-derived organoids (PDOs) typically monitored in bright-field mode. The software was tested on diverse datasets, including 91 organoid images. It outperformed existing tools such as CellProfiler and OrganoSeg in segmentation accuracy, as evaluated by metrics including Jaccard distance and precision, while maintaining competitive processing speeds (Fig. 3c).

The versatility of MOrgAna has been demonstrated across multiple organoid types and imaging platforms, from confocal microscopy of human brain organoids to high-content screening of gastruloids. It successfully quantified subtle morphological changes in brain organoids over time using parameters such as area and perimeter. It also introduced lobe contribution elliptical Fourier analysis (LOCO-EFA) to capture complex shape dynamics without manual annotation. The software processed large-scale datasets, such as a 0.5 TB time-lapse gastruloid dataset, within hours, requiring only minimal training data (approximately 1% of the images). Its modular design and user-friendly interface cater to both novice and advanced users, offering manual curation to refine results. By automating segmentation and quantification without labeling biases, MOrgAna provides a robust and scalable solution for standardizing organoid analysis, which will enhance the reproducibility of biomedical research.

Organoids exhibit complex structural features, including shape, size, internal organization, and the presence of specific biomarkers, all of which correlate with their functional status. MOrgAna allows automated quantification of morphological and fluorescence data across multiple imaging systems, improving image analysis throughput while reducing manual intervention. Similarly, deeper learning methods, such as U-Net-derived pipelines, have been used to resolve the intricate phenotypes of 3D organoids with high precision for segmenting complex structures, such as cerebral organoid ventricles [61]. Furthermore, novel voxel-based unsupervised feature-learning frameworks, such as Phindr3D, have strong potential for extensive phenotypic profiling of organoid populations, enabling advanced clustering and functional classification tasks.

At the tissue level, AI has transformed and refined histological data for improved diagnostics. Innovations such as generative adversarial networks allow the transformation of cryosectioned images into formalin-fixed paraffin-embedded (FFPE)-like images, significantly improving image quality and facilitating intraoperative diagnostic assessments without compromising clinically relevant features [61]. Similarly, the development of interpretable deep learning pipelines, such as im4MEC, has extended AI applications to molecular classification tasks, bridging morphology and molecular phenotyping to refine clinical stratifications with high accuracy [57]. This fusion of tissue-level morphological and molecular insights is a substantial advancement in AI-enabled pathology analyses.

As organoid research delves further into multiomics, the high dimensionality and heterogeneity of datasets challenge conventional methods of data integration and mining. AI overcomes these hurdles through scalable multi-modal integration of biological data. For example, UnitedNet is used to synthesize RNA, DNA, and protein data for high-precision single-cell analysis [62]. Likewise, devCellPy offers an automated machine learning pipeline that identifies complex, multi-layered cellular annotations in single-cell transcriptomic datasets, raising the standards for high-resolution functional annotation [63]. These methods improve data interpretation across diverse omics modalities and reveal biological relationships that were previously inaccessible.

AI-driven approaches have also been adopted to track transplanted organoids in vivo for longitudinal, quantitative insights. For example, combining magnetic particle imaging with machine learning algorithms (e.g., *k*-means) enables precise monitoring of stem cell-derived islet organoids in transplantation models [64].

3.3 AI-enabled organoids in preclinical applications

AI-enabled organoid research has also advanced preclinical applications. Researchers have developed novel systems by integrating AI with organoid technology to accurately mimic human physiological and pathological conditions, revolutionizing disease modeling, drug discovery, and precision medicine [65]. This synthesis of computational and biological methods offers opportunities to analyze complex biological processes, enhance the precision of drug testing, and combat the inefficiencies of conventional models. This subsection emphasizes various applications of AI-driven organoid systems, which are categorized as core research areas.

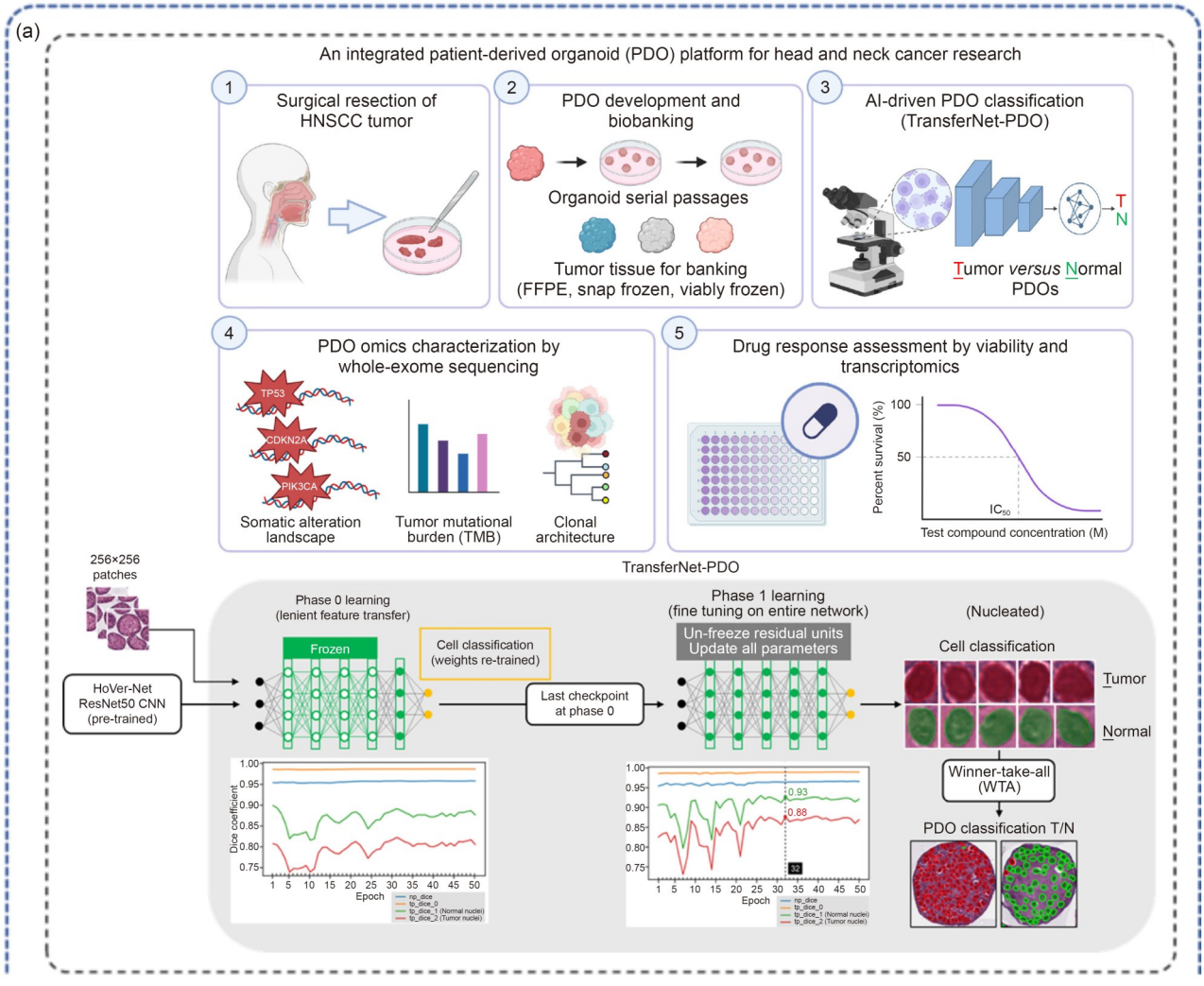
3.3.1 Disease modeling, diagnosis, and risk prediction

AI-enabled organoids pave the way for more accurate and predictive disease models, particularly through machine

learning and computational tools. These platforms enable researchers to model disease progression, optimize clinical decision-making, and predict treatment outcomes [26]. One key example of AI's application in early disease detection is in research on pancreatic cancer, a malignancy with a poor prognosis when diagnosed in later stages. To detect pancreatic cancer sooner, Placido et al. [66] developed a deep learning algorithm trained on clinical data from diverse populations, showing promise for predicting disease onset up to three years before clinical diagnosis. This model, called CancerRiskNet, achieved an area under the receiver operating characteristic curve of 0.88 for patients in a Danish cohort, highlighting the potential of longitudinal datasets and machine learning in risk prediction.

In personalized disease modeling, PDOs are integrated with AI to simulate patient-specific conditions. Monzel et al. [67] developed machine learning algorithms to analyze midbrain organoids derived from patients with Parkinson's disease (PD), quantifying dopaminergic neurons and neuronal complexity. These analyses provide valuable insights into PD progression and allow for neurotoxicity assessments in response to compounds such as 6-hydroxydopamine [67]. To provide a comprehensive and scalable system for translational cancer research, Doerfler et al. [68] combined AI-driven digital pathology and genomics to establish PDOs as a novel alternative model for drug response in cancer. Here, PDOs were created from surgically resected head and neck squamous cell carcinomas and monitored by a new CNN model, TransferNet-PDO, that accurately distinguished tumors from normal PDOs using digital histopathology images. This integrated PDO platform combines organoid establishment, tissue biobanking, multiomics characterization, AI-driven histopathological classification, and functional drug screening (Fig. 4a).

Computational modeling of cardiac organoids is advancing our understanding of congenital defects, as shown by Feng et al. [69], who used machine learning to study hiPSC-derived heart organoids harboring mutations in the Ebstein's anomaly-linked *NKX2-5* gene. By computationally profiling the transcriptomic and structural landscapes of



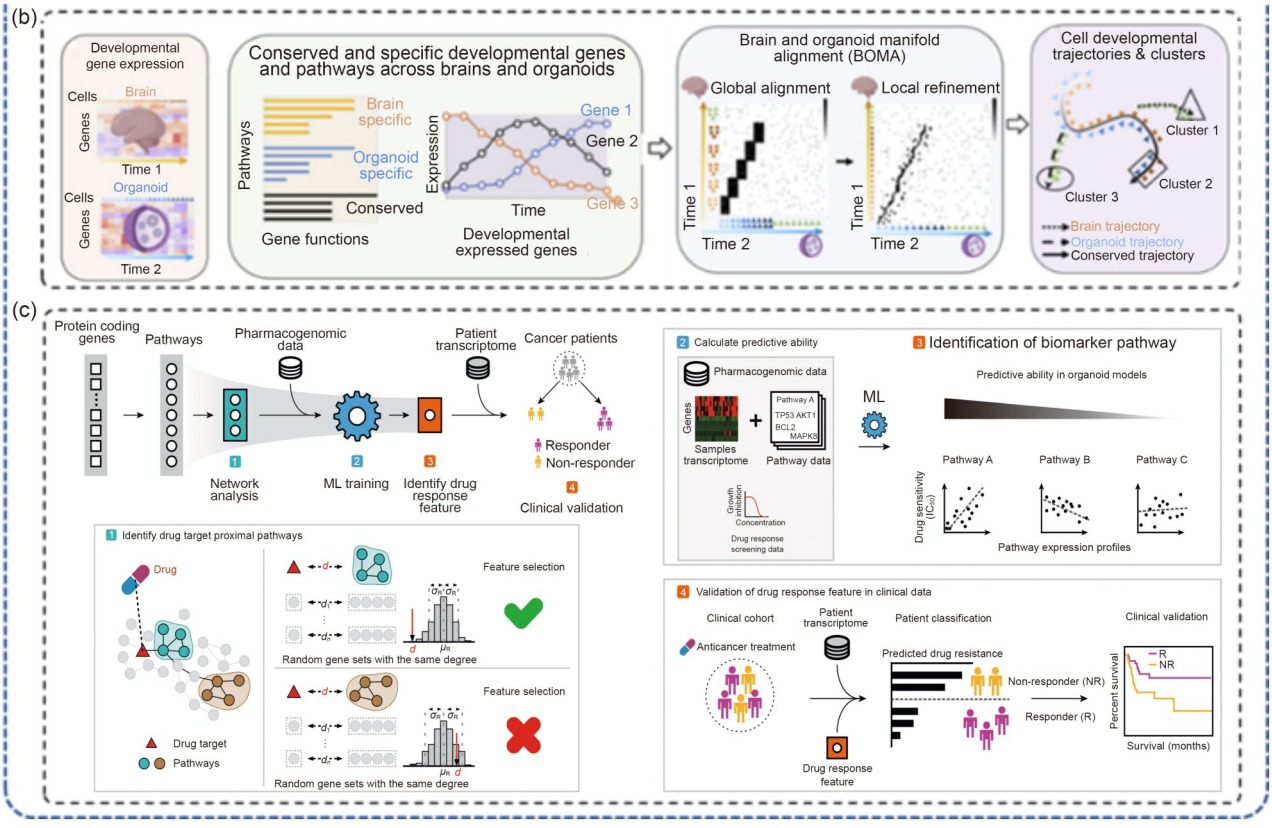


Fig. 4 Illustration of artificial intelligence (AI)-driven optimization of organoid models. (a) Integrating AI-driven digital pathology and genomics to establish a convolutional neural network (CNN) TransferNet-PDO model for drug response in cancer. Reproduced from [68], licensed under CC BY-NC-ND. (b) Global and local developmental gene expression alignment of human and non-human primate brains and organoids using brain and organoid manifold alignment (BOMA). Reproduced from [74], licensed under CC BY-NC-ND. (c) Identification of biomarkers associated with drug response using a network-based machine learning approach. Reproduced from [75], licensed under CC BY 4.0

these organoids, this study recapitulated congenital defects at the atrialized ventricular level, creating realistic models for rare and understudied cardiac diseases. Beyond direct organoid models, AI-enhanced prediction tools such as OncoNPC are also bridging gaps in cancer of unknown primary diagnostics. By training on next-generation sequencing data from over 36,000 tumors, OncoNPC precisely identified cancer origins and improved outcomes for patients whose AI-guided treatments aligned with their tumor profiles. This framework highlights the power of combining genetic datasets with machine learning to enhance diagnostic accuracy in clinical oncology [70].

3.3.2 Organoid biobanks and precision medicine

The integration of AI tools with living organoid biobanks is advancing precision medicine through systematic storage, utilization, and analysis of diverse organoid data [71]. Through patient-derived tumor organoids, biobanks are an invaluable resource for studying tumor heterogeneity, genotype-phenotype relationships, and therapeutic responses. Jacob et al. [72] established a glioblastoma (GBM) organoid biobank that recapitulated the cellular and mutational heterogeneity of parental tumors. This platform facilitates the rapid testing of patient-specific therapies, including chimeric antigen receptor T-cell immunotherapies, and is a robust model for understanding tumor evolution and aggressiveness. Similarly, living biobanks derived from patients with breast cancer were able to preserve the hormonal receptors and histopathological and genetic characteristics of the original tumors, demonstrating their utility in both in vitro and in vivo studies [73].

He et al. [74] introduced the brain and organoid manifold alignment (BOMA), a machine learning framework for comparing gene expression profiles between human brains and organoids, with implications for organoid biobanks and precision medicine. BOMA consists of two steps: global alignment of developmental gene expression data based on time, followed by local refinement using manifold learning to map conserved and divergent developmental trajectories at cellular resolution. When applied to bulk and single-cell RNA sequencing (scRNA-seq) datasets, BOMA revealed that human cortical organoids align more closely with

cortical brain regions than with non-cortical areas, identifying region-specific gene expression programs. It also identified the conserved trajectories across human and non-human primate brains near birth, alongside organoid- and brain-specific cell clusters, which were validated experimentally via immunofluorescence for key differentially expressed genes (Fig. 4b).

The utility of the machine learning framework for organoid biobanks lies in its ability to integrate and analyze heterogeneous scRNA-seq data from multiple studies, reducing batch effects and experimental confounders such as culture conditions. BOMA identified developmental similarities and disparities, such as fewer superficial-layer neurons in organoids than in the brain, which can help guide organoid protocols to better mimic human brain development; it can also establish PDO biobanks tailored for precision medicine by identifying cell type-specific genes and pathways for tailored drug screening, rendering BOMA an invaluable resource for leveraging organoid models in clinical research.

Researchers can tap into biobanks better thanks to AI, which streamlines complex datasets to identify new correlations. For example, using an AI-assisted morphological typing system for colorectal cancer (CRC) organoids, researchers have shown that specific morphologies are linked to key cellular pathways, such as cell adhesion and ribosome biogenesis. These insights were subsequently validated using targeted inhibitors, thereby opening new avenues for cancer therapeutics [22].

3.3.3 Drug testing and discovery

Organoid technology, coupled with AI, is improving the speed and accuracy of preclinical drug screening and evaluation for the design of more personalized therapeutics. For example, Kong et al. [75] developed a machine learning framework whose network-based analysis can identify robust biomarkers for morphological drug testing and discovery using pharmacogenomic data from 3D organoid models of colorectal and bladder cancers. This study aimed to predict patients' responses to 5-fluorouracil (5FU) and cisplatin by identifying drug sensitivity markers in organoid-derived transcriptomic data. The approach identified the "activation of BH3-only proteins" pathway as a key determinant of 5FU response in CRC organoids, linking such expression to apoptosis initiation and DNA damage response; this finding was validated by survival predictions in 114 patients. Similarly, for cisplatin in bladder cancer, elevated expression of the "amino acid synthesis and interconversion" pathway correlated with sensitivity, as confirmed in 77 patients and external isogenic cell line datasets, suggesting metabolic influences on drug efficacy (Fig. 4c).

Unlike conventional machine learning methods, this network-based model enhances predictive accuracy and interpretability by embedding a protein–protein interaction network to reduce biological complexity and capture robust signals. The framework outperformed standard approaches in classifying patient-specific drug responses and was further validated with somatic mutation-based biomarkers. When applied to organoids, it elucidated morphological and molecular features tied to drug sensitivity without requiring extensive pretreatment molecular data. However, future integration with datasets such as Library of Integrated Network-based Cellular Signatures (LINCS) L1000 could refine predictions. By focusing on organoid pharmacogenomics, this study provided a scalable method for biomarker discovery directly applicable to morphological drug testing, which could accelerate personalized cancer therapy development.

AI-powered image segmentation tools such as attention and cross U2Net (AU2Net) and res-double dynamic conv attention U-Net (RDAU-Net) have significantly advanced the analysis of organoid-based drug screening. In bladder cancer research, AU2Net enhances segmentation accuracy by integrating highly specific deep learning modules, thereby refining evaluations of drug-treated organoids. This model achieved an F1-score of 91.54%, surpassing conventional U-Net-based analysis [76]. Similarly, RDAU-Net optimizes high-throughput organoid drug screening by including advanced convolutional and attention mechanisms for more precise evaluation of treatment efficacy [77].

Applying AI to pharmacogenomic datasets has also helped pinpoint therapeutic biomarkers. Kong et al. [75] applied network-based machine learning tools to colorectal and bladder cancer organoids, accurately predicting drug responses based on pharmacogenomic markers. Such models enable scientists to fine-tune therapies based on detailed organoid genotype-phenotype mapping. Moreover, the comboFM machine learning framework has made significant progress in predicting the synergistic effects of drug combinations. With tensor factorization to analyze sparse preclinical datasets, comboFM identified novel combinatorial therapies for precision oncology applications, including the synergistic pairing of anaplastic lymphoma kinase (ALK) inhibitors (e.g., crizotinib) and proteasome inhibitors (e.g., bortezomib) for lymphoma [78].

Various tissue techniques have been advanced in recent years, alongside the growing demand for blood–brain barrier modeling and toxicity evaluation for drug development. Bell et al. [79] explored a method using blood–brain barrier organoids for high-throughput toxic export. Conversely, Zhang et al. [80] conducted an extensive quantitative morphological analysis of drug responses to PDOs using optical coherence tomography. The method performed organoid segmentation and multi-parameter quantitative

analysis using the deep learning network (EGO-Net), with the quantitative results validated against adenosine triphosphate (ATP) assays and integrated into a morphological index for drug-response assessment. Compared with single-time-point morphological parameters, time-varying morphological parameters more accurately reflected pharmacodynamic changes.

Organoid imaging for drug screening has advanced through AI-enabled analytics. Fillioux et al. [81] developed a novel imaging-driven high-throughput screening strategy that leverages time-lapse microscopy recordings of PDOs to dynamically quantify real-time drug efficacy by incorporating SAM segmentation algorithm and DI-NOv2 model into a unified processing pipeline. They embedded a dedicated attention mechanism into a multi-instance learning architecture to aggregate complementary spatial and temporal features for ATP concentration prediction with superior predictive performance compared with conventional time-agnostic approaches. Tan et al. [82] developed DILITracer, a deep learning framework trained to predict drug-induced liver injury (DILI) levels from bright-field images of human liver organoids. By leveraging a BEiT-V2 vision transformer pretrained on 700,000 cell images, the model utilized transfer learning to better extract spatial and temporal features from organoid morphologies. This approach allows for accurate classification of compounds into clinically defined DILI categories, such as hepatotoxic risk.

3.4 Limitations of AI-enabled organoids

Despite the transformative potential of AI in optimizing organoid development, several limitations must be considered. First, the performance of AI models relies heavily on the quantity, quality, and representativeness of training datasets. Currently, most AI-driven organoid optimization studies rely on small-scale, institution-specific datasets that lack diversity in cell sources, tissue origins, and experimental conditions. This data bias can lead to overfitting, and the related models are difficult to generalize to novel organoid systems or clinical samples, limiting their translational applicability. Second, many advanced AI architectures are difficult to interpret, slowing the iterative refinement of organoid protocols due to their "black-box" nature; they rarely provide mechanistic insights into why specific parameters drive organoid maturation or functionality, although they can help identify optimal parameter combinations (e.g., growth factor ratios, biomaterial properties) [83]. The precision of optimization outcomes may also be limited in existing AI models that rely on static or semi-static data inputs, and fully capturing the dynamic and spatiotemporal complexity of organoid development, including cell–cell interactions, matrix remodeling, and temporal changes in signaling pathways, remains challenging.

4 AI-enabled organoids/OoC

The integration of AI into OoC systems enables precise modeling of human organ function while addressing the challenges posed by the vast datasets generated by these systems [84, 85]. However, analyzing the complex and high-throughput data output from OoCs places significant demands on conventional data processing methods, warranting the integration of AI with OoCs that mimic the physiological and pathological conditions of human tissues and organs. Deep learning and machine learning approaches, including natural language processing, CNNs, and reinforcement learning, are now indispensable tools for automating data analysis and enabling real-time insights into cellular behavior in OoCs [85, 86].

4.1 Data analysis and predictive modeling using AI

One of the biggest advantages of AI integration into OoC systems is its capability to analyze vast datasets and resolve complex multidimensional relationships. Conventional machine learning algorithms, such as SVMs and random forests, have helped models predict drug efficacy and toxicity from experimental data. For example, one study used a range of machine learning algorithms across over 5000 datasets to identify drug-target properties [87]. Moreover, AI methods, such as DeepH-DTA, have employed hybrid architectures that combine bidirectional ConvLSTMs for drug sequences and dense CNNs for protein interactions to predict drug-target affinities with unprecedented accuracy, helping repurpose drugs, such as those for COVID-19 [88].

The combination of deep learning and microfluidics is promising for predicting cell behavior parameters and monitoring cell cultures from OoC images. Pérez-Aliacar et al. [89] developed a CNN-based methodology for real-time parameter identification from fluorescence images of GBM cell cultures in microfluidic devices, thereby advancing AI-driven data analysis and predictive modeling. The CNN was trained on synthetic data generated from a validated mathematical model, incorporating three key parameters (proliferation rate, chemotaxis coefficient, and hypoxia threshold) that define the "go or grow" behavior of GBM, critical for prognosis and treatment response. The trained network achieved high accuracy (Pearson's ρ>0.99) in predicting these parameters, robustly cross-validated and filtered noise, and generalized effectively to conditions beyond the training set. When applied to a real microfluidic experiment image, the CNN accurately extracted parameters to rapidly characterize GBM dynamics [89] (Fig. 5).

This approach integrates deep learning with microfluidics for real-time analysis, a significant advance over conventional

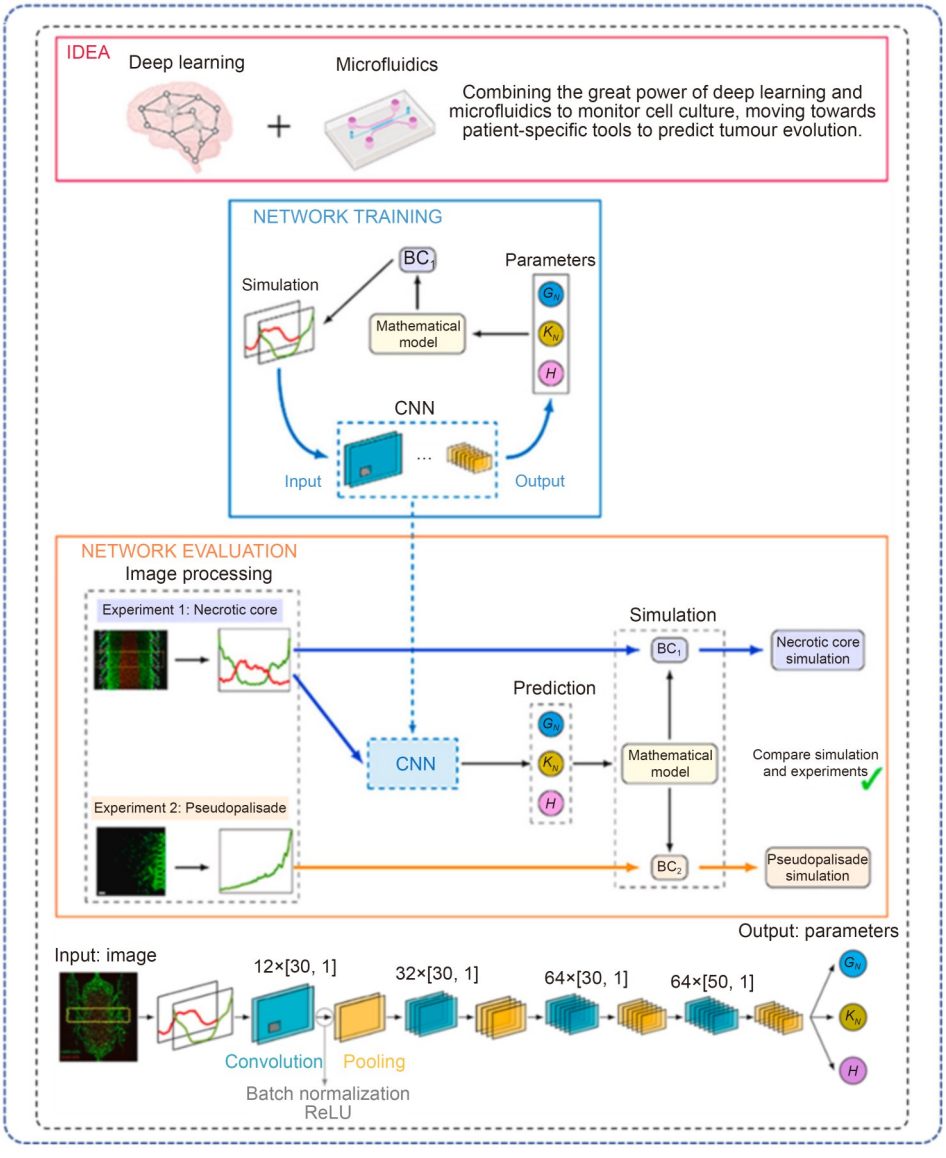


Fig. 5 Illustration of artificial intelligence (AI)-enabled organoids and organ-on-a-chip (OoC). Cell culture monitoring and parameter identification in glioblastoma (GBM) using convolutional neural networks (CNNs). Reproduced from [89], licensed under CC BY-NC-ND

fitting methods, as it eliminates the need for patient-specific parametric adjustments after training. While limited to predefined parametric forms and specific experimental setups (e.g., necrotic core formation), this study highlights the potential of AI-driven tools for patient-specific tumor forecasting. By combining CNN predictions with controlled in vitro cultures, it lays the foundation for scalable predictive modeling in organoid research, namely personalized GBM treatment strategies.

Advanced predictive models elucidate tissue responses to experimental conditions. Nguyen et al. [90] demonstrated that AI-powered tumor-on-a-chip systems could reveal immune cancer dynamics at the system level, including real-time visualization of antibody-dependent cytotoxicity. Additionally, the incorporation of AI tools into OoCs has enormous potential for rare disease research, where patient-specific models can identify therapeutic strategies, unlike conventional preclinical models [85].

4.2 Enhancements in imaging and tracking capabilities

AI has significantly enhanced cellular imaging and tracking within OoC platforms. Deep learning models such as CNNs and U-Net architectures can achieve semantic segmentation of cellular images and precise tracking of dynamic phenomena [91, 92]. Parlato et al. [93] demonstrated a tumor-on-a-chip system that offers accurate real-time analysis of dendritic cell migration toward tumor cells by combining advanced microscopy with deep-learning-based

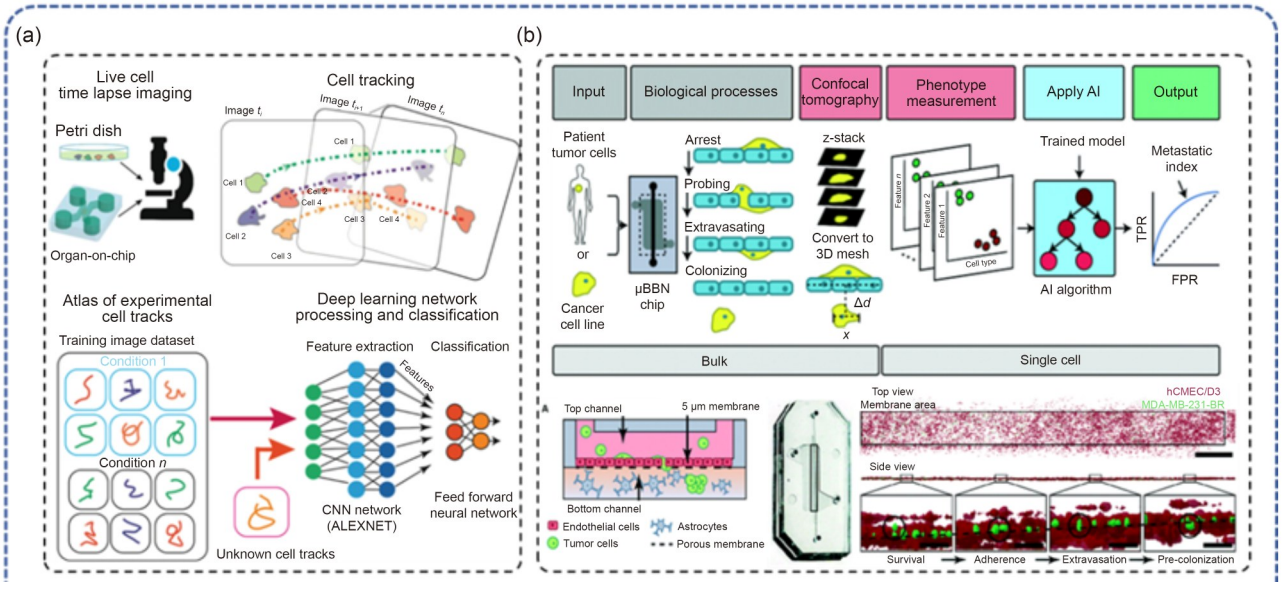
tracking algorithms. AI has also shown its utility in quantifying cellular trajectories of dendritic cell behavior and drug effects within this system.

Deep-learning-assisted in vitro evaluation of cancer drug treatments can reveal novel insights on cell trajectories. For example, Mencattini et al. [91] introduced "Deep Tracking," a deep-learning-based method to enhance imaging and tracking capabilities for automated cell motility analysis using time-lapse microscopy images. This approach generates a visual atlas from single-cell trajectories, encodes motility descriptors, and employs a pretrained CNN to extract high-level features for classification. This was validated in two scenarios: immune cells co-cultured with breast cancer cells in 3D biomimetic gels within OoC devices under immunotherapy and clustered prostate cancer cells in Petri dishes treated with chemotherapy. In both cases, Deep Tracking accurately classified trajectories based on drug presence, achieving superior performance compared with conventional one-dimensional (1D) deep learning methods and basic feature classifiers, which struggle with track length heterogeneity and limited feature generality (Fig. 6a).

The innovation of this method lies in its ability to handle diverse track lengths, ranging from 23 h on average in 72-h experiments to 6 h in 6-h observations. Importantly, it does this without the need for artificial padding, which helps maintain the temporal integrity of the data and reveals a universal "motility style." When applied to organoids, it tracks complex cellular interactions such as immune cancer cell dynamics and collective cancer cell responses with high fidelity. By leveraging 2D trajectory representations, Deep Tracking overcomes the limitations of prior approaches while offering robust scenario-agnostic analyses. This study demonstrated the potential for real-time high-throughput tracking in organoid systems, providing a scalable tool for decoding motility patterns and enhancing imaging-based insights into drug effects and cellular behaviors.

Similarly, Nguyen et al. [90] used AI tools on an $HER2^+$ tumor-on-a-chip to explore multicellular interactions among cancer cells, immune cells, and cancer-associated fibroblasts. This platform revealed how fibroblasts can suppress the effectiveness of immunotherapy, illustrating the ability of AI to enhance OoC analysis in complex scenarios. For skeletal muscle cells on a chip, Jena et al. [94] integrated RNNs and CNNs to analyze dynamic cell repositioning and morphological changes. This emphasizes AI's promising role in understanding tissue biomechanics and designing targeted therapeutic strategies.

Furthermore, AI enhances cellular imaging and tracking within OoC platforms by integrating confocal tomography with neural networks. Oliver et al. [95] developed a microfluidic blood–brain niche (μBBN) platform, combined with advanced imaging and AI, to enhance tracking and imaging capabilities for assessing the potential of cancer cell extravasation into the brain. By integrating confocal tomography with a neural network, they explored the behavior of triple-negative breast cancer cell lines, including MDA-MB-231 and brain-seeking MDA-MB-231-BR, as well as patient-derived xenografts (PDXs) of different tumor origins. Cancer cells were seeded onto a human cerebral microvascular endothelial layer within the μBBN device, imaged for 24–48 h using confocal microscopy, and analyzed for four parameters: percent volume extravasated, distance traversed, sphericity, and cell volume. The AI algorithm, trained on these metrics, distinguished brain metastatic cells from nonmetastatic cells, achieving a positive predictive value of 0.91, with an area under the curve (AUC) of 0.95 for cell lines and 0.97 for PDXs, outperforming other classifiers such as random forest and logistic regression.



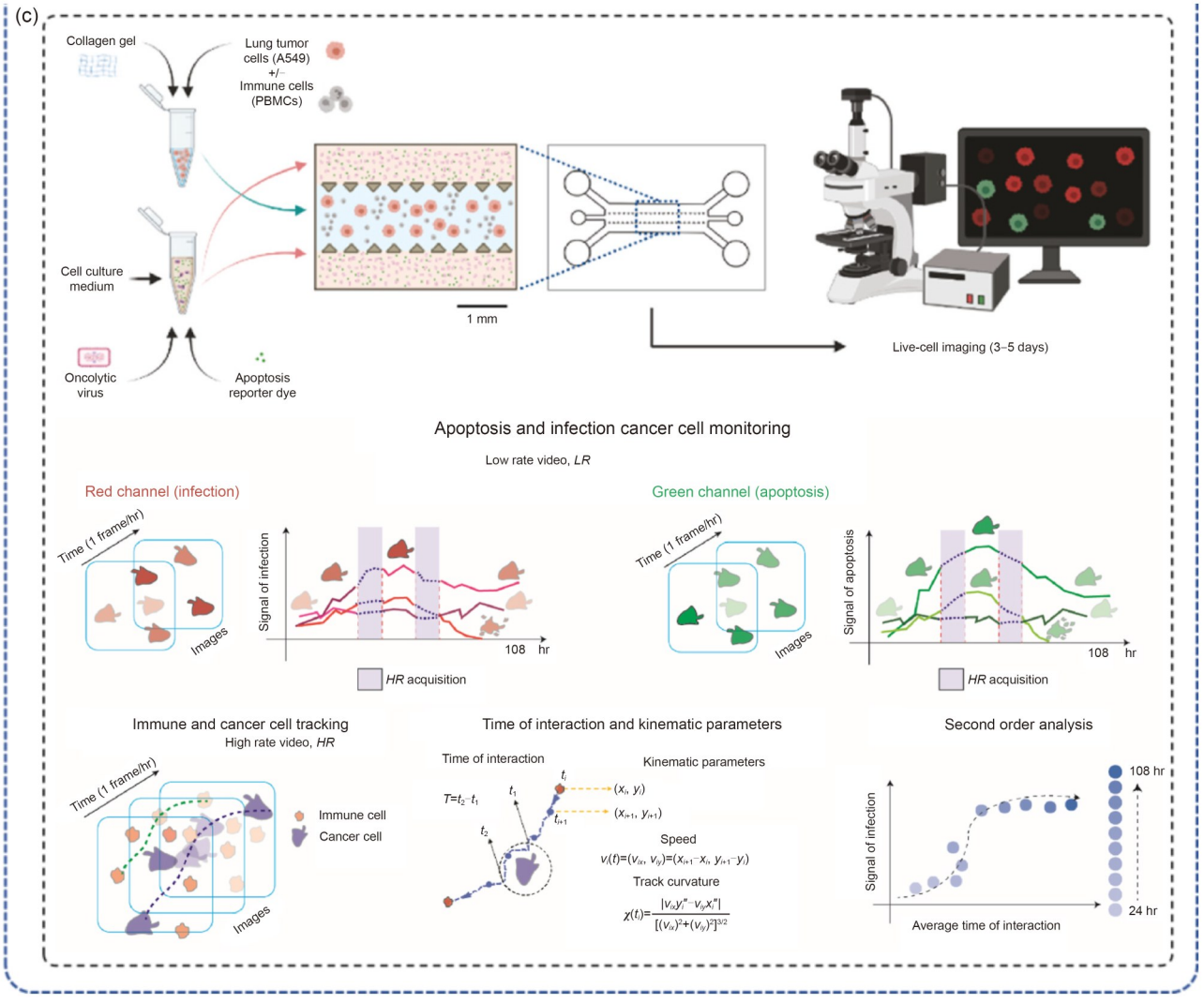


Fig. 6 Representative applications of artificial intelligence (AI) in cancer-on-a-chip and in vitro cancer models. (a) Schematic of deep learning-assisted in vitro evaluation of cancer drug treatments to discover novel messages within cell trajectories. Reproduced from [91], licensed under CC BY 4.0. (b) Microfluidic blood–brain niche (μBBN) device design and AI-based identification of the extravasation potential of cancer cells into the brain metastatic niche. Reproduced from [95], with permission from The Royal Society of Chemistry. (c) Cancer cell infection and killing by oncolytic vaccinia virus with immune cells using a tumor-on-chip approach. Reproduced from [99], with permission from Elsevier B.V.

This platform provides a robust, AI-enhanced method for real-time, high-resolution tracking of metastatic potential in organoid-like systems for personalized diagnostics (Fig. 6b).

Additionally, customized AI imaging systems, such as those utilizing Anderson-localizing optical fibers, have effectively overcome the limitations of conventional imaging by providing artifact-free, real-time visualization over long distances [96]. Furthermore, deep-learning-based reconstruction frameworks have visualized the 3D tomography of cellular structures, such as red blood cells, by handling ill-posed imaging problems with fidelity, thus widening the scope of computational optical imaging applications in OoCs [97].

4.3 Automation and optimization in iOoC systems

iOoC systems can leverage AI to automate labor-intensive steps for more complex analyses. Deep learning allows near-instantaneous adaptation to experimental deviations through real-time monitoring of cellular biomarkers within iOoCs [98]. Cell motility analysis in tumor microenvironments has also benefited from automation as shown by Mencattini et al. [91], whose novel deep learning approach generates a "motility style" atlas of cell movement using time-lapse microscopy data, assuring highly accurate and unbiased classification of drug-treated and untreated cells [91].

This automated framework offers scalability for future OoC studies while maintaining high accuracy. Mencattini et al. [99] investigated the mode of action of the oncolytic vaccinia virus (OVV) in cancer immunotherapy by combining tumor-on-a-chip technology with image analysis. They analyzed time-lapse videos of cancer-immune co-cultures within a tumor-on-a-chip model by developing automated algorithms. Real-time feedback systems in iOoCs provide a closed-loop control system for enhanced experimental reproducibility by dynamically monitoring and adjusting experimental parameters using embedded sensors. Real-time monitoring of OVV infection and cancer cell death is achieved by quantifying parameters such as cancer cell infection, apoptosis levels, immune cell speed, trajectory curvature, and cancer-immune interaction time. Their results revealed a synergy between OVV and immune cells that induced massive tumor cell apoptosis within four days (Fig. 6c).

This study implemented a second-order analysis to correlate infection levels with immune cell kinetics, demonstrating that OVV infection increased immune cell speed and track curvature by Day 2, followed by extended interaction times and reduced speed by Day 3, indicative of an immune attack on infected cells. This automation reduces bottlenecks in manual analysis and enhances throughput and precision in tracking dynamic 3D microenvironments, and it ultimately revealed a cause-and-effect relationship between cancer cell infection and immune recognition. Although limited to apoptosis detection, this approach provides a scalable framework for integrating additional cell death markers and will streamline immunotherapy research and development within iOoC platforms.

Another application of machine learning-enabled screening is probiotics. In this space, Wu et al. [100] developed an intelligent intestine-on-a-chip integrating a machine learning system to screen for relief-enteritis functional probiotics. They fabricated a high-throughput microfluidic chip integrated with environmental control systems that provided a standardized, scalable intestinal microenvironment for multiple probiotic co-cultures. An intestine-on-a-chip was constructed to evaluate the interactions between 12 *Bifidobacterium* strains and host cells of the colitis model with accuracy, completeness, and efficiency. The most effective contender was found to relieve intestinal inflammation and enhance epithelial barrier function in vitro and in vivo; it can intelligently distinguish minor therapeutic variations in probiotic strains and prioritize their efficacy, allowing economical, efficient, and accurate functional probiotic screening (Fig. 7a).

4.4 Translational applications of AI-OoC integration

OoCs have transformed preclinical drug evaluation by replicating human organ-level functions. Combining OoCs with AI further enhances their utility by streamlining pharmacological screening. OoC systems that integrate deep learning models, such as DeepCell, have shown efficacy in predicting drug toxicity and pharmacokinetics by combining liver-on-a-chip models with AI to simulate drug metabolism and improve the predictions of adverse drug effects [85]. Similarly, AI-assisted OoC studies for immuno-oncology drug testing have revealed novel insights on the efficacy of therapies, such as trastuzumab, in targeting $HER2^+$ tumors [90].

AI-powered OoCs are advanced tools for studying disease pathophysiology. AI can construct personalized OoC models for precise simulations of rare or neglected diseases by analyzing patient-specific data, as shown by Lefebvre et al. [98], who used AI algorithms to segment and track subcellular structures within OoC systems and generated insights into mitochondrial dynamics, which are crucial for understanding various metabolic diseases. Personalized medicine can benefit from AI-enhanced OoCs that enable patient-specific organ simulations [88]. Additionally, AI-equipped drug-on-chip platforms can forecast patients' responses to different treatments using integrated multiscale data, benefiting immunotherapy and targeted cancer treatments [90].

A digital twin is a virtual modeling technology that integrates computer simulations and experimental data [101]; it creates digital replicas of organs or physiological systems by converting in vitro data obtained from organoids/OoC MPS to predict behavior and performance and assess the distribution, metabolism, and safety of drugs. In this way, Graf et al. [102] demonstrated a three-organ (gut–liver–placenta) MPS combined with a digital twin framework to evaluate the pharmacokinetics of prednisone and its primary active metabolite, prednisolone. Prednisone crosses the placental barrier when combined with physiologically-based pharmacokinetic (PBPK) modeling during pregnancy, and the transfer of prednisolone is limited by the blood–placental barrier on the fetal side. These protective mechanisms reduce fetal exposure to potentially nontoxic levels of prednisolone. They simulated oral drug administration and tracked drug metabolism and transplacental transfer to translate the MPS-generated data into human physiology. The results showed that digital twinning, closely aligned with the experimental data generated by the MPS system, could support early-stage drug safety assessments for vulnerable populations (Fig. 7b).

Interconnected "human-on-a-chip" systems, comprising multiple microfluidic OoCs, are a significant advance in predictive modeling of systemic drug effects and inter-organ interactions. Augmented by AI, these platforms can accurately model physiological processes such as metabolism, drug absorption, and organ crosstalk. Automated modular systems have been developed to

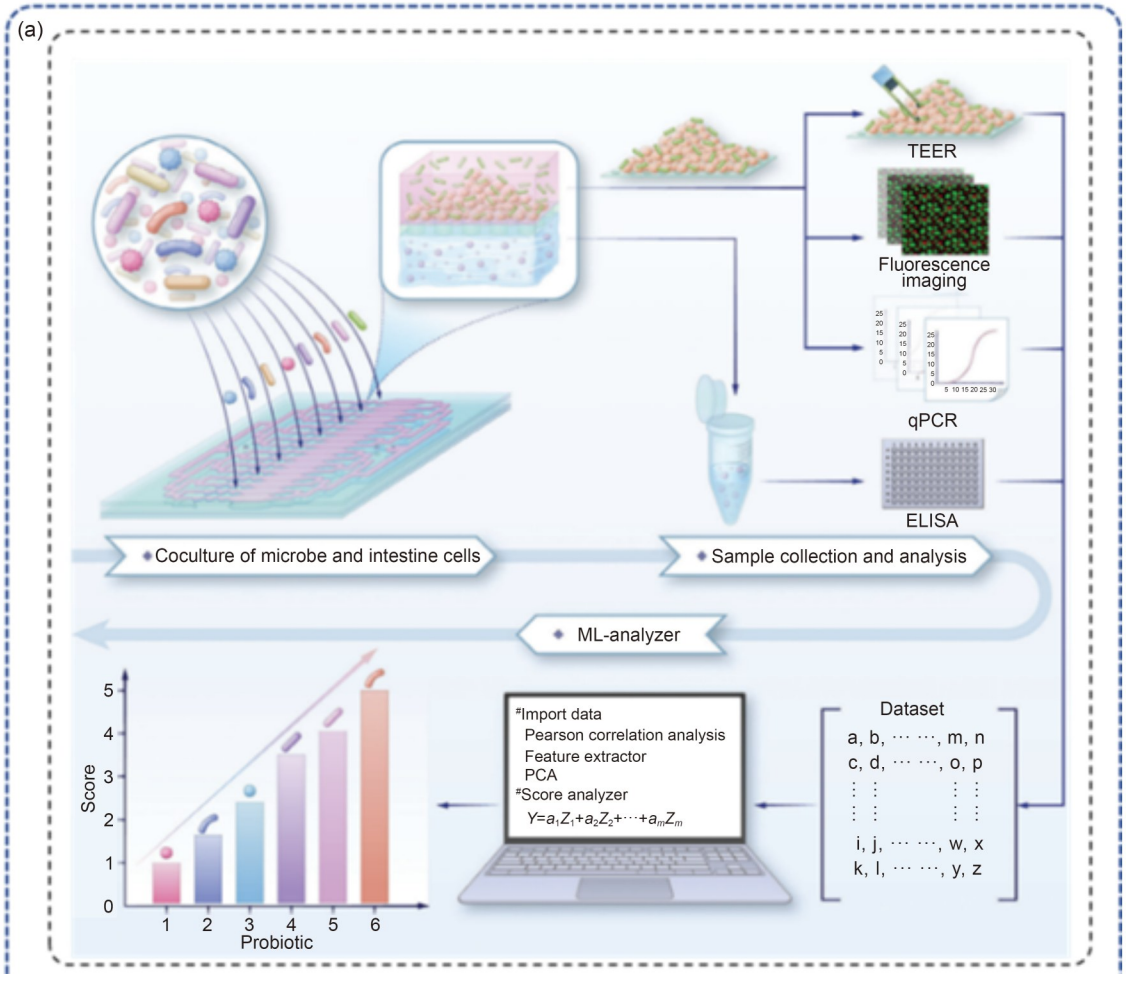
maintain multiple organ-specific conditions for extended periods [97].

4.5 AI-assisted decision-support systems (DSSs)

AI-enabled systems have shown promise in personalized medicine and clinical decision-making by integrating insights from diverse modalities, such as PDO data, genetic profiles, and phenotypic drug responses [64]. AI-assisted DSSs are now a critical bridge between organoid/OoC technology and clinical translation applications [103]. These systems integrate machine learning algorithms with multi-modal data streams from organoid cultures (e.g., transcriptomic profiles, morphological phenotypes, and drug response kinetics) and OoC platforms (e.g., tissue barrier function metrics and real-time physiological signals) to generate data-driven recommendations for clinical practice [104]. In oncology, AI-DSS can analyze PDO responses to a panel of chemotherapeutic agents, correlating drug efficacy with genomic biomarkers to predict patient-specific treatment outcomes. By leveraging supervised learning to train large cohorts of PDO-OoC data linked to clinical outcomes, these systems enable clinicians to prioritize therapies with the highest likelihood of efficacy while minimizing adverse effects, enhancing the precision of treatment selection for diseases such as cancer, neurodegenerative disorders, and rare genetic conditions [105]. Moreover, AI-DSS facilitates real-time adaptation of treatment strategies. As longitudinal data from patient-matched organoids/OoCs are fed into the system, reinforcement learning algorithms can refine predictions to account for changes in disease progression or treatment resistance, offering a dynamic decision-making tool for personalized treatment optimization [106].

4.6 Limitations of AI-enabled organoids/OoC systems

AI-enabled organoid/OoC systems are constrained by several limitations in their ability to model human physiology. The primary challenge lies in integrating multimodal, high-dimensional data generated by OoC platforms, including metabolomics, transcriptomics, imaging,



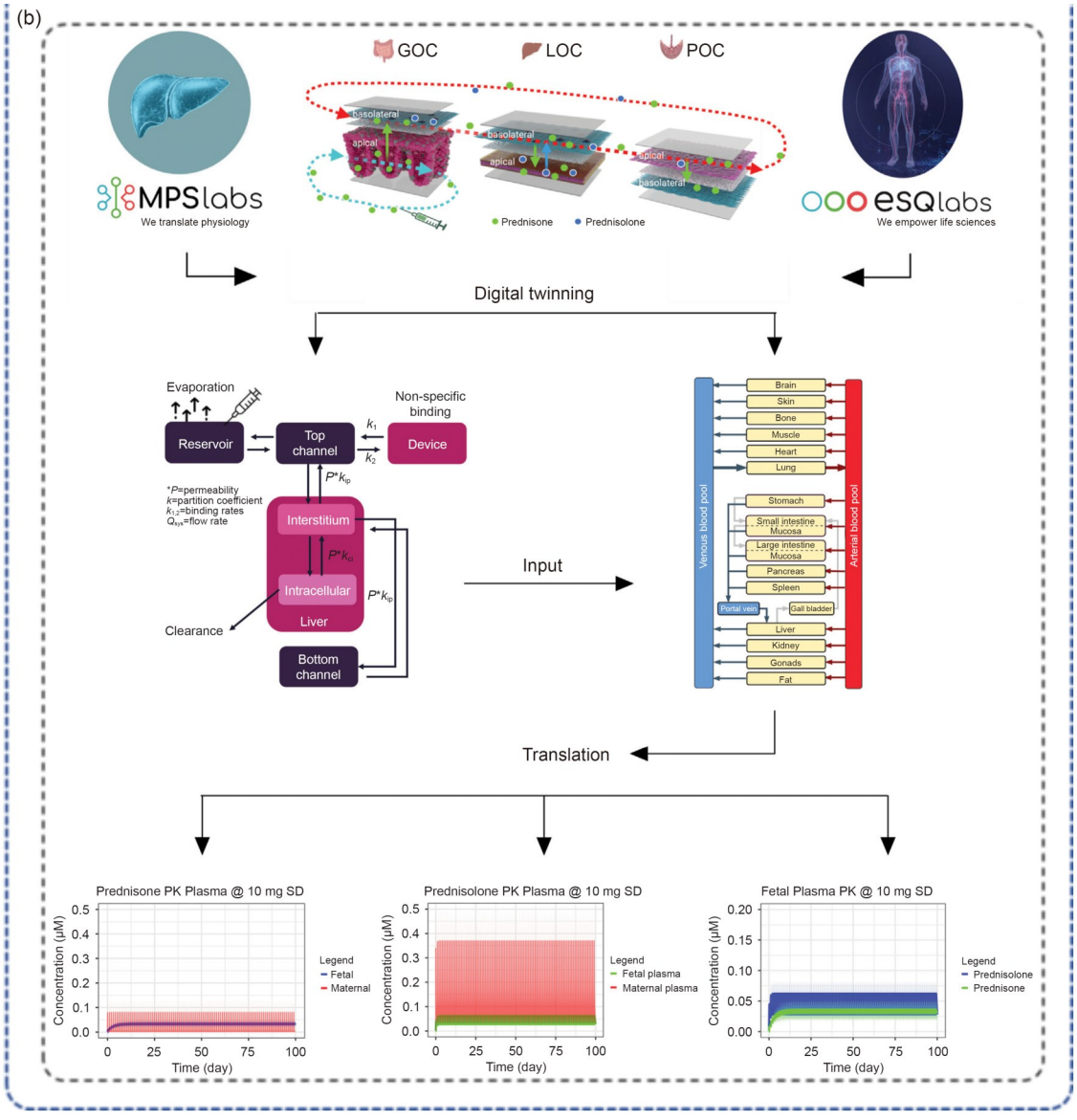


Fig. 7 Artificial intelligence (AI)-enabled intelligent organ-on-a-chip (iOoC) systems for data analysis and optimization. (a) An intelligent intestine-on-a-chip integrating machine learning to screen relief-enteritis functional probiotics. Reproduced from [100], with permission from Wiley-VCH GmbH. (b) Three-organ (gut–liver–placenta) microphysiological system (MPS) combined with a digital twin framework to measure the pharmacokinetics of prednisone and its active main metabolite, prednisolone. Reproduced from [102], licensed under CC BY 4.0

and electrophysiology [107]. Current AI models often excel at single-modality data analysis but struggle to effectively harmonize heterogeneous datasets, leading to incomplete characterization of organoid/OoC functionality and missing correlations between biological phenotypes. Moreover, the scalability of AI models is limited by complex data integration and reproducibility challenges, and is further hindered by the lack of standardized data acquisition protocols across OoC platforms owing to variations in imaging resolution, sensor sensitivity, or culture conditions. Another critical limitation is the gap between the complexity of in vitro models and the physiological relevance of in vivo models [108]; AI-derived insights could be biased toward in vitro-specific phenotypes. AI algorithms optimize data analysis based on the signals generated by OoC systems. However, these models may still fail to recapitulate key in vivo features, especially systemic immune interactions and vascularization. Additionally, the real-time AI integration with OoC systems remains underdeveloped for most research-grade OoC platforms; their dynamic feedback loops are hindered by computational latency and the need for robust, low-power hardware integration. Finally, the ethics and regulation of AI-driven predictive models remain debatable, given concerns about data privacy, model validation, and the liability for AI-generated recommendations based on organoid/OoC data [109].

5 AI-enabled integrated workflow for organoids and OoC

AI-enabled OoC systems follow a sequential, integrated workflow, as shown in the diagram, with three core modules: inputs/data sources, AI engine, and outputs/value. (Fig. 8). First, multi-modal experimental data such as imaging, multiomics, sensor-derived physiology, and microfluidic parameters are aggregated to capture the spatiotemporal, molecular, and biophysical complexities of organoids/OoCs. These data are processed by a multifunctional AI engine that performs perception (segmentation/tracking of organoid structures), representation (integrating heterogeneous data via embedding/graph networks), prediction (modeling pharmacodynamics/toxicology), and control (adaptive optimization of culture parameters via closed-loop reinforcement learning). Critically, the engine is anchored by three quality pillars: standardization, credibility, and interpretability, which ensure the rigor of the AI outputs. Finally, these outputs enhance the realism of organoid/OoC models by recapitulating an in vivo-like architecture, boosting efficiency through high-throughput screening/automated analysis, and enabling personalization from PDO responses.

This framework shows how AI bridges organoid/OoC technology and practical applications, addressing the key limitations of conventional in vitro models. This system overcomes the fragmentation of conventional analysis by consolidating diverse high-dimensional organoid/OoC data streams into a unified AI-processing pipeline to capture biological behaviors comprehensively. The AI engine's dual focus on functional processing (perception and prediction) and quality assurance (standardization and interpretability) ensures that the outputs are both biologically meaningful and methodologically robust. The AI-enabled workflow advanced translational research, and the resultant value propositions of more realistic, efficient, and personalized organoids/OoCs addressed the unmet needs in drug discovery and precision medicine.

6 Conclusions

AI is shifting paradigms in high-throughput screening for organoid and OoC technologies. These intelligent systems leverage machine learning and data analytics to optimize experimental design, interpret complex biological data, and improve the predictive accuracy of drug candidates and toxicity risk assessments. AI models can integrate multi-source data, including genomics, metabolomics, and clinical information, to build predictive frameworks that complement or

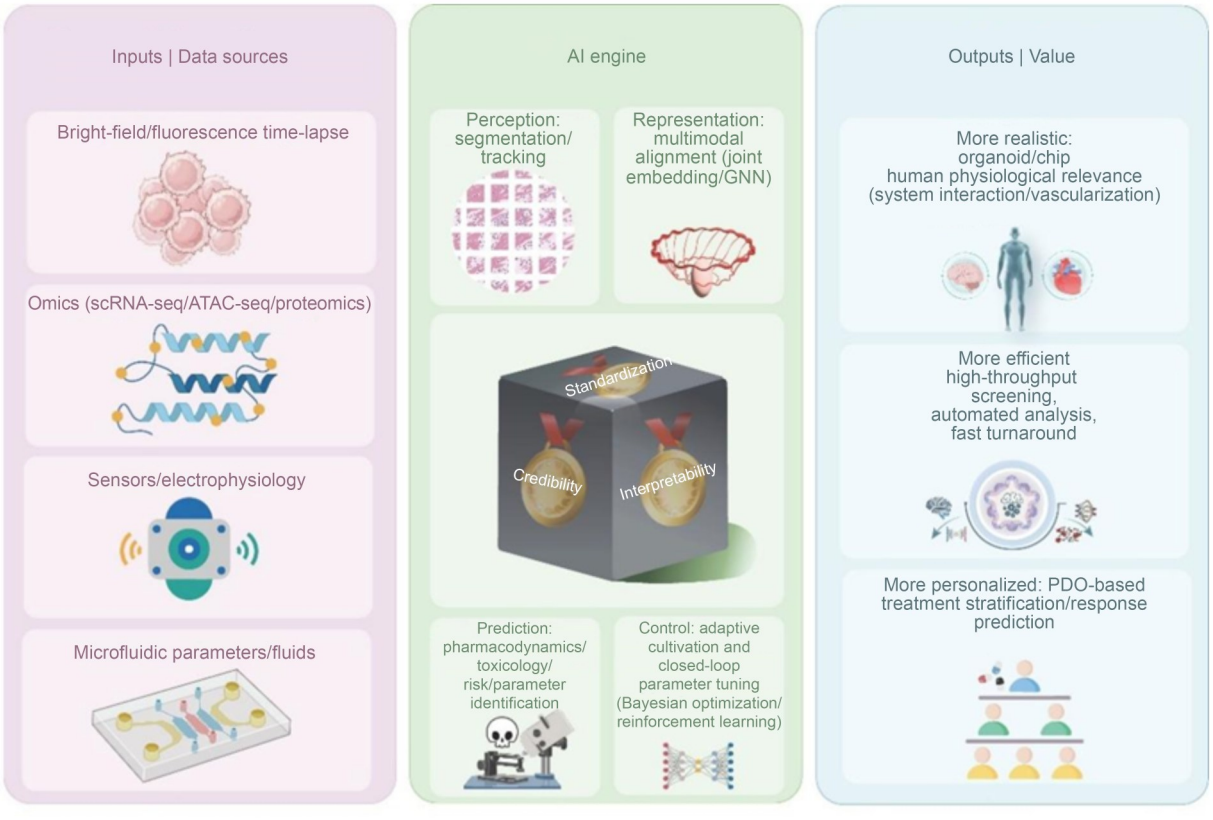


Fig. 8 The integrated workflow of artificial intelligence (AI)-enabled organoid and organ-on-a-chip (OoC) systems with three core modules: inputs/data sources, AI engine, and outputs/value

even replace certain phases of animal testing. Moreover, intelligent AI agents facilitate automation of organoid cultivation and analysis, thereby improving consistency and scalability. AI can generate "digital twin" models from PDOs to simulate individualized drug responses and therefore enhance precision in personalized medicine treatment planning. They are transforming into early-stage drug screening by offering more humanized, efficient, and cost-effective alternatives due to their inability to fully replace animal models, especially for systemic toxicity and immune response validation. Regulatory bodies such as the Food and Drug Administration (FDA) have already begun incorporating data from these AI-integrated platforms into clinical trial applications, signaling a shift toward more human-centric drug evaluation.

Future research will prioritize enhancing vascularized platforms using hydrogel scaffolds or endothelial co-culture methodologies within OoC systems. Emerging computational paradigms, particularly AI, are increasingly applied to manage expansive datasets generated by integrated OoC platforms. AI-enabled image analysis, coupled with deep learning algorithms, allows for concurrent real-time monitoring and high-content data mining, thereby enhancing the detection of subtle cellular responses to external stimuli. The integration of computer vision technologies into OoC architectures can revolutionize pharmaceutical screening paradigms, enabling high-throughput compound evaluations within abbreviated timelines [110]. Furthermore, deformable mesh microelectronics demonstrate bidirectional interfacing capabilities between bioelectronic systems and organoid models, with applications in neural stimulation and pathological modeling [111].

The transformative capabilities of AI are becoming increasingly evident. AI-driven algorithms can rapidly interpret multiomics datasets, facilitating advances in personalized therapeutics and accelerating drug discovery. These systems exhibit unparalleled efficiency in processing high-dimensional data while identifying complex biological patterns and correlations beyond conventional analytical methodologies. Furthermore, AI-integrated organoid platforms optimize experimental workflows, improve reproducibility, and offer economical solutions for pathological modeling and pharmacological assessments [27].

As AI algorithms evolve, integrating multiomics datasets with advanced imaging and organoid modeling will accelerate the development of personalized therapeutics. The FDA Modernization Act 2.0 supports the use of new approach methodologies (NAMs), including organoids, OoC systems, computational models, and in vitro human cell systems, as alternatives to certain animal studies [24]. NAMs include organoids, OoC, computer models, and in vitro human cell systems. Future AI developments should prioritize transforming opaque computational frameworks into biologically interpretable white-box models to better elucidate underlying physiological mechanisms. Implementing unified multi-modal analytical platforms with cross-institutional compatibility has amplified the translational value of organoid-based investigations. Through the seamless integration of spatiotemporal resolution and functional omics profiling, AI methodologies have the potential to establish predictive models for organoid morphogenesis, developmental trajectories, and functional dynamics. However, standardization hurdles persist in AI-enabled organoid research, including protocol heterogeneity, inconsistent model generalizability, and the scarcity of large-scale curated datasets. Ethical considerations regarding genomic data privacy and equitable access to biobanks require collaboration between researchers, clinicians, and policymakers.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

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