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Review

Next-generation discovery: empowering organoid research with machine learning, artificial intelligence, and mathematical modeling

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Organoids have rapidly matured into powerful model systems. The field is pushing organoids toward architectural sophistication and functional fidelity, with longitudinal experiments producing ever-larger and more complex datasets. As a result, computational methods have become indispensable for experimental design, data analysis, and predictive modeling, as well as for obtaining mechanistic insights. In this review, we survey recent progress at the interface of organoid research and computational approaches, discuss key challenges on both fronts, and outline future directions to maximize impact in biomedical research through convergent, synergistic efforts.

Highlights

Machine learning/artificial intelligence-guided monitoring and robotics during organoid culture ramp-up, stabilizes, and homogenizes organoid models.

Machine learning/artificial intelligence approaches can extract meaningful patterns even when experimental data are noisy, incomplete, or heterogeneous.

Mechanistic mathematical models and artificial intelligence-guided experimental design enable *in silico* perturbations and facilitate concrete, experimentally verifiable hypotheses that accelerate organoid research.

Computational methods integrate and interpret large-scale datasets, advancing clinical translation and therapeutic applications.

Organoid culture and phenotypic characterization

With the advent of organoid technology, applications across the life sciences have expanded rapidly. Organoids provide a versatile, physiologically faithful alternative to traditional (cancer) cell lines, while reducing reliance on animal models (Box 1). Organoids can be cultured in scaffold-free formats (e.g., air–liquid interface and bioreactors) or embedded in extracellular matrix (ECM) to mimic native stem cell niches (Figure 1). Current work is pushing these systems toward greater biological fidelity by increasing cellular diversity, refining spatial organization, and improving microenvironmental control (see reviews by Kim *et al.* [15] and Artegiani and Hendriks [16]). In parallel, characterization is becoming deeper and more continuous. Image-based readouts remain foundational: snapshot microscopy (confocal and wide field) captures morphology and, together with staining, detects a limited panel of proteins under steady-state conditions, whereas live cell imaging yields rich time series. The increasing complexity of such image data requires advanced computational analysis; deep learning-based **segmentation** (see Glossary) and tracking have become central for extracting single-cell (sc) information, and Table 1 summarizes available tools in diverse organoid applications. Beyond imaging, multiomics assays—proteomics, metabolomics, lipidomics, and bulk RNA-seq—support molecular classification, while sc technologies (e.g., scRNA-seq, snRNA-seq, and scATAC-seq) provide transcriptomic and epigenomic profiles at sc resolution [59,60]. Most recently, **spatial omics** methods link high-dimensional molecular information to spatial context, enabling the identification of spatially organized cellular niches, and are particularly valuable in organoid systems [26,61]. Together, these advances generate large, heterogeneous, and longitudinal datasets that cannot be analyzed manually. Computational approaches are therefore essential—not only for managing and interpreting high-dimensional data but also for supporting experimental design, gaining mechanistic insights, and enabling predictive modeling.

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Box 1. Organoids—a concise overview.

Organoids are 3D self-organizing multicellular entities that recapitulate many aspects of the native organ and are often referred to as 'mini organs'. Organoids can be derived from embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells (ASCs):

ESC-derived organoids: Pluripotent ESCs emerge from the inner cell mass of the blastocyst during early stages of development. ESCs are differentiated under embryonic signaling cues to the different germ layers (ectoderm, endoderm, and mesoderm) to generate organoids resembling distinct organs. ESC-derived organoids are predominantly used for the study of organ development [1]. Using such strategies, researchers have successfully established ESC-derived organoids for a variety of tissues, including the lung [2], mammary gland [3], cerebral [4], inner ear [5], and retina [6]. However, ESCs are derived from embryonic tissue, which raises ethical concerns when obtained from human sources.

iPSC-derived organoids: iPSCs are generated by reprogramming adult somatic cells through the introduction of the pluripotency-associated transcription factors OCT4, SOX2, KLF4, and c-MYC, collectively known as the Yamanaka factors [7]. The plasticity of iPSCs enables the generation of a wide range of tissues and developmental stages, making iPSC-derived organoids a model for studying early development, disease mechanisms, and genetic disorders [8]. iPSCs can be differentiated into one or more of the three different germ layers by manipulating stem cell-relevant pathways, such as the WNT, transforming growth factor β, and fibroblast growth factor signaling pathways: the endoderm (gastrointestinal tract and respiratory tract), the mesoderm (kidney, muscles, heart, and blood vessels), and the ectoderm (nervous system, eyes, and ears). iPSC-derived organoids have been established from multiple tissue types, including the cerebral [1], lung [9] and kidney [143]. Major challenges of iPSC-derived organoid generation are cellular heterogeneity and incomplete maturation.

ASC-derived organoids: Tissue-resident ASCs play a key role in maintaining tissue homeostasis and regeneration. Sato *et al.* [10] discovered that embedding ASCs of the murine intestine in extracellular matrix and exposing them to their natural niche factors results in the spontaneous formation of organoids that are characterized by asymmetric stem cell proliferation and differentiation. Patient-derived organoids (PDOs) are highly relevant for the modeling of diseases and for the study of tissue homeostasis. However, PDOs are restricted to the epithelial compartment and lack essential components such as stroma, nerves, immune cells, and vasculature [11]. Personalized PDOs can also be generated from cancer stem cells after tumor resection, making them an attractive model for personalized therapy [12]. Several PDO biobanks have been established, including breast [13], lung [14], and colorectal [12] cancers.

In this review, we survey recent advances at the interface of organoid research and computational sciences, highlight current challenges, and outline future directions toward more sophisticated organoid systems. We focus on studies in which computational modeling or computer-driven analysis materially shaped the scientific outcome.

Developmental biology and tissue homeostasis

Organoids are used to address central questions of developmental biology [induced pluripotent stem cell (iPSC)- and embryonic stem cell (ESC)-derived organoids] [62], as well as tissue homeostasis and organization [patient-derived organoids (PDOs)] [11]. Quantitative imaging underpins these studies: machine learning (ML)/artificial intelligence (AI)-based software tools (Table 1) segment and track cells, assess neighborhood structures, and quantify (morphological) characteristics of organoids. As input data, they use bright-field and fluorescence images and work in one or multiple planes [32,34,42]. Recent developments focused on the label-free tracking of single cells [63], cell tracking algorithms, including the prediction of annotation errors via a probabilistic graph [43], assessment of organoid morphology and topology [33], the reconstruction of whole 3D organoids from a few confocal planes [44], and the noninvasive classification of **differentiation** states based on Raman confocal microspectroscopy [64]. Robustness can be further improved by harmonizing size and shape: combining micropatterning with AI analysis revealed calcium transient upstroke as a key determinant of cardiac organoid geometry [65].

Image-derived features increasingly inform mechanistic models. Early work used **continuum model** formulations to capture transport fields (e.g., nutrients), to move boundaries (e.g., organoid surfaces) [66], and to study self-organization [67]. Hybrid approaches that integrate

Glossary

Batch/noise structure: systematic, nonbiological variation in organoid imaging or omics data arising from differences in culture conditions, experimental platforms, or automation, which can affect ML/AI model performance if unaccounted for.
Cell-resolved model: a computational model that explicitly represents individual cells and their interactions. Uses formalisms such as agent-based models, where cells are treated as discrete agents with rule-based behaviors; vertex models, where tissues are represented as networks of connected cell–cell interfaces; or cellular Potts models, where cells are modeled as deformable regions on a lattice governed by an energy functional.
Continuum model: mathematical description using partial differential equations to model spatial gradients of nutrients, stresses, or signaling molecules within organoids.
Counterfactual simulation: use of calibrated models to explore hypothetical perturbations to test causality and guide experiment design.
Data integration: combination of multimodal datasets (imaging, single-cell, and spatial omics) to jointly infer structure, state, and function of organoids.
Differentiation: the process by which stem cells acquire specialized identities; studied in organoids to mimic tissue development and disease progression.
Federated learning: privacy-preserving training of shared models across distributed datasets without transferring raw data, supporting multicenter organoid studies.
Homogeneous model: a mathematical framework describing whole-organoid dynamics using averaged variables such as size or cell number.
Hybrid model: combines discrete cellular representations with continuous environmental fields to link single-cell behavior to tissue-scale phenomena.
Identifiability: the ability to uniquely determine model parameters from experimental data, ensuring interpretable and reliable mechanistic inference.
Joint latent space: a shared low-dimensional representation learned, for example, by an autoencoder, from multiple data modalities [e.g., images and (spatial) omics] in which

multiscale mathematical simulations with experimental perturbations have reproduced crypt-like patterning in intestinal organoids consistent with Turing-type mechanisms [68]. Complementary analyses of cell–cell communication quantify microenvironmental impacts [69]. A dedicated pipeline now streamlines image-based modeling, **parameter estimation**, and **uncertainty quantification** [70].

Organoid differentiation can be assessed using transcriptomics to complement image-based readouts. The development of an unsupervised ML-based alignment algorithm, BOMA, has enabled comparative analysis of gene expression between brains and organoids [71]. Using this tool, researchers have revealed spatiotemporal and cross-species gene expression patterns hidden in transcriptomic data. However, many approaches require the characterization of specific cell types at the sc level and with spatial resolution, which demands multimodal approaches. Albanese *et al.* [72] introduced SCOUT (Single-cell and Cytoarchitecture analysis of Organoids using Unbiased Techniques), an automated multiscale imaging and analysis platform that integrates high-resolution 3D reconstruction with a U-Net-based convolutional neural network (CNN) to extract comprehensive molecular, cellular, and cytoarchitectural features. Applied to multiple organoids, SCOUT mapped cytoarchitecture, correlated ventricle size with protein expression, identified rare cell populations, and quantified Zika virus-induced alterations in organoid morphology and composition.

Concluding, characterizing organoid morphology and deciphering cell differentiation are essential for investigating mechanisms of development and tissue homeostasis. Computational analyses facilitate the integration and interpretation of complex multimodal data, whereas computational modeling serves as a key framework for generating and testing biological hypotheses.

Disease models and personalized therapies

Organoids provide physiologically relevant systems to elucidate disease mechanisms, identify therapeutic targets, and develop personalized therapies. Models exist for diverse conditions, including cystic fibrosis (CF) [57] and cancers [12]. Recent years have witnessed advancements in disease modeling, especially through the incorporation of CRISPR gene editing [73]. Complementary CRISPR perturbation screens generate high-dimensional datasets [74], where ML algorithms are already used to improve gRNA predictive accuracy across Cas variants [75], and provide optimized prime editing design using transfer learning for nucleotide language models [76]. Sahoo *et al.* [77] applied a Boolean network explorer-based ML approach to link transcript-inferred disease progression continuum states in inflammatory bowel disease (IBD) with disease outcome, identifying gene clusters critical for maintaining the epithelial barrier, and when perturbed in intestinal organoids, result in a disease model for IBD. Simulation frameworks further reduce experimental burden (e.g., brain organoid models of Alzheimer's disease [78]). Multiomics integration links morphology to mechanism and response: MOFA (multiomics factor analysis) associated colorectal cancer organoid size with pathway activities (Insulin-like growth factor 1 receptor (IGF1R) vs. WNT) and therapy sensitivity (Mitogen-activated protein kinase (MAPK) vs. WNT inhibitors) and connected a gene set to intertumoral heterogeneity [79].

Computational methods also refine treatment response assessment. In cancer organoids, traditional PDO drug sensitivity assays rely on endpoint bulk ATP measurements, which lack single-organoid and temporal resolution. Several software tools are available to predict organoid viability using bright-field images [45,47,48]. A drug response metric (normalized organoid growth rate) in combination with an ML-based image analysis (OrBITS, Organoid Brightfield Identification-based Therapy Screening) provides richer information from transmitted light microscopy, distinguishing cytostatic from cytotoxic effects [49,80]. Other software tools enable the prediction of apoptosis [81] and provide accurate single-organoid growth dynamics [46,82] (Table 1). This, in turn, allows

corresponding entities are encoded in a common coordinate system to enable integrated analysis and comparison.
Mechanochemical instabilities: pattern formation or structural changes that emerge from feedback between biochemical processes and mechanical forces in tissues or organoids.
Microfluidic systems: miniaturized systems enabling precise control of flow, gradients, and stimuli; used for dynamic organoid culture and real-time monitoring.
Parameter estimation: inference of quantitative model parameters from data using optimization to align predictions with observations.
Segmentation: computational delineation of organoid or cellular boundaries in microscopy or spatial omics data, forming the basis for quantitative morphological analysis.
Spatial omics: molecular profiling techniques that preserve positional information, enabling the mapping of cellular states, gradients, and niches in organoids.
Uncertainty quantification: an estimation of confidence in model parameters or predictions to avoid overinterpretation and support decision-ready outputs.

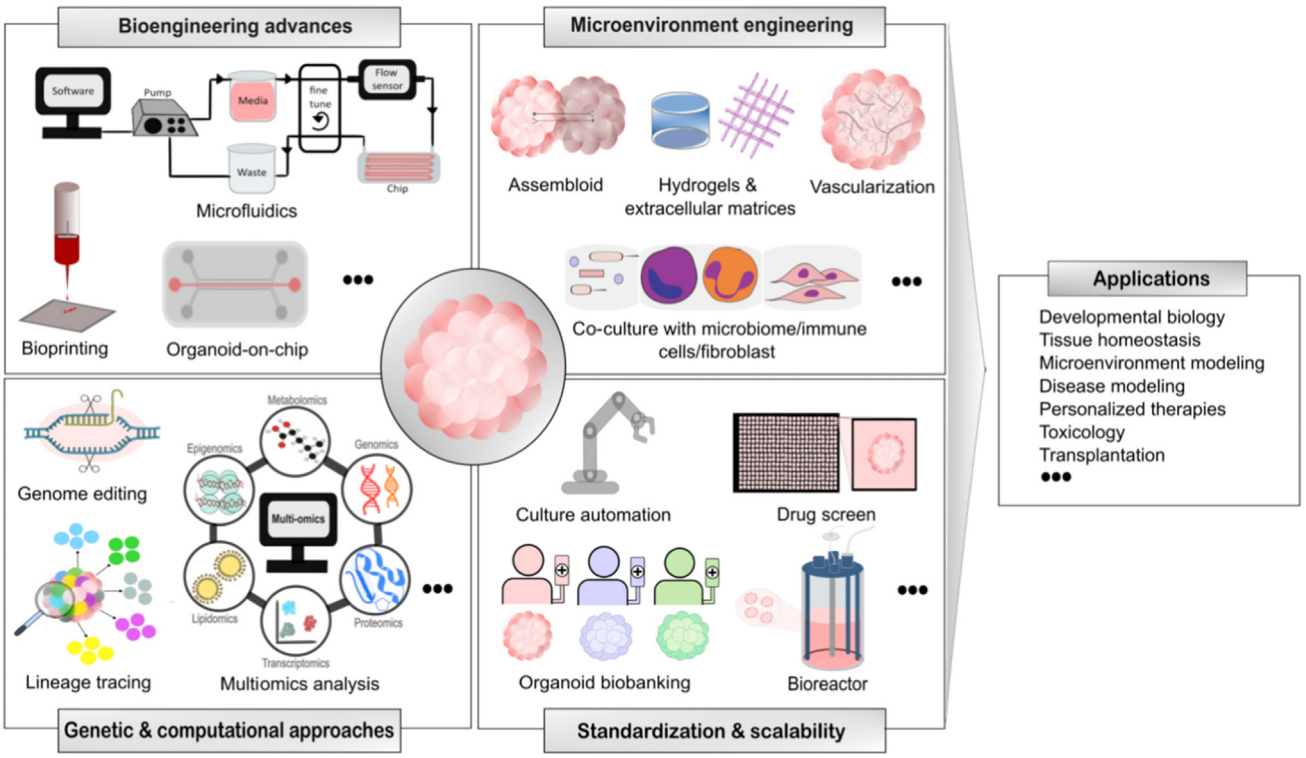


Figure 1. Experimental advances and applications of organoids. (Left) Schematic overview of four key developmental tracks: bioengineering advances, microenvironment engineering, genetic and computational approaches, and standardization and scalability. (Right) Application areas of organoids. AI: artificial intelligence; ML: machine learning.

for patient-specific mathematical modeling of growth dynamics [83]. CellNEST, a graph neural network, integrates spatial transcriptomic data of pancreatic ductal adenocarcinoma PDOs that link tumor-promoting communication patterns to disease progression, treatment response, and patient outcomes [84]. Oliver *et al.* [85] used neural networks on a 3D blood–brain barrier model with primary human cells and astrocytes to distinguish GFP-labeled cancer cells capable of forming brain micrometastases from those that could not. The model accurately predicted the metastatic potential of cancer cells. Variance in the data continues to pose a persistent challenge for computational approaches. In an effort to reduce interexperimenter data variability, studies implemented organoid bioprinting and ML-based monitoring [86,87]. Additional standardization leverages micropatterned materials to harmonize organoid size and shape and improve ML classifiers in screening, for example, neuruloids for therapies against Huntington's disease [88].

Organoids hold the promise to revolutionize personalized medicine, and integrating ML with emerging experimental platforms offers a powerful path toward personalized medicine: Park *et al.* [89] showed that a random forest classifier is able to predict tumor regression after radiotherapy with an accuracy of 89% based on the radiotherapy resistance of colorectal cancer PDOs. Ji *et al.* [90] and Zhou *et al.* [91] demonstrated that the integration of cancer cell line transcriptomics and PDO drug sensitivity data with the chemical structures of anticancer drugs using an AI framework enables effective prediction of patient clinical outcomes. Furthermore, Kong *et al.* [92] showcased that ML approaches can be used to determine drug biomarkers based on pharmacogenomic data from colorectal and bladder cancer PDOs. Advances in technical

Table 1. Overview of computational organoid image analysis

Image analysis tool	Applied to	Field of application	Imaging technique	Z-resolved	Segmentation	Tracking	Specialty/notes	Refs
CellPose	Cancer [17–21], brain [22–24], colon [25,26], and other [27–31]	Diverse	Fluorescence	✓	✓	×	Single-cell extraction; no retraining required	[32]
3DCellScope	PDAC organoids	Disease modeling	Fluorescence (nuclei and membrane)	✓	✓MS	×	Assessment of organoid morphology and topology	[33]
MOrgAna	Gastruloids [34–38], brain [34,39], and other [34,40,41]	Development and tissue homeostasis	Bright-field and fluorescence	✓	✓	×	Broad application; flexible input data	[34]
D-CryptO	Intestinal organoids	Development and tissue homeostasis	Bright-field	×	✓	×	Organoid maturation assessment	[42]
OrganoidTracker 2.0	Intestinal organoids	Tissue homeostasis	Fluorescence (nuclei)	✓	✓	✓	Fully automated analysis	[43]
VONet	Colon and colon cancer organoids	Tissue homeostasis and disease modeling	Fluorescence (nuclei)	✓	✓MS	×	3D structure from minimal confocal stacks	[44]
Ins-ATP	Diverse cancer organoids	Disease modeling	Bright-field	×	×	×	Estimates ATP from bright-field images	[45]
OrganoID	Diverse cancer organoid	Disease modeling	Bright-field and phase-contrast	×	✓	✓	Labeling and single-organoid tracking during treatment	[46]
deepOrganoid	Colorectal cancer PDOs	Disease modeling	Bright-field	×	✓	×	Drug sensitivity prediction	[47]
OrgaExtractor	Colon cancer organoids	Disease modeling	Bright-field	×	✓	×	Drug sensitivity; correlates to ATP readouts	[48]
OrBITS	PDAC [49–53], NSCLC [49,52], matched normal [49], and melanoma [54]	Disease modeling	Bright-field	×	✓	✓	Drug sensitivity; correlates to ATP readouts	[49]
Organoid App	Extrahepatic organoids	Coculture modeling	Bright-field	×	✓	×	Identification in dense cocultures	[55]
OrganoIDNet	PDAC organoids	Disease modeling	Bright-field	✓	✓	✓	Size-specific drug sensitivity; eccentricity	[56]
DETECTOR	Colorectal organoids (patients with CF)	Disease modeling	Bright-field	×	Density map	×	Drug sensitivity prediction	[57]
OrgaSegment	Colorectal organoids (patients with CF)	Disease modeling	Bright-field	×	✓	×	CFTR-mediated organoid swelling analysis	[58]

Check marks indicate which features are covered, while crosses indicated features which are not covered. No reference in 'Applied to' column indicates tool reference as application reference.
Abbreviation: MS: multiscale segmentation method.

screening platforms, such as closed-loop **microfluidic** systems, combined with ML-based **data integration**, enable the identification of optimal durations, doses, and sequences of clinically used combinatorial drug treatments, offering valuable insights to guide future therapeutic regimens [93]. In the future, technological innovations may shorten the time from tumor resection

to organoid-based drug testing for efficient personalized therapy. For example, micro-organospheres, generated *in situ* from patient tumor tissue using droplet microfluidics, can be integrated with an AI-driven image analysis pipeline to enable differentiation between cytotoxic and cytostatic effects, as well as discrimination of drug responses originating from PDOs versus stromal components [94]. Extending beyond cancer, ML models such as DETECTOR [57] and OrgaSegment [58] enable detailed analysis of bright-field images of rectal and intestinal organoids from patients with CF, enabling automated prediction of therapy sensitivity across diverse genetic variants. Clinical trials have been conducted stratifying patients with CF based on intestinal organoid response to different cystic fibrosis transmembrane conductance regulator (CFTR) modulators (clinical trial: NTR7520).

Drug-specific pharmacokinetics and toxicity remain key bottlenecks in drug development. Policy shifts (e.g., FDA Modernization Act 3.0[i]) encourage human-based models and *in silico* approaches, providing the basis for the use of organoid and organ-on-chip systems. These platforms capture human-relevant biology and yield rich phenotypes [95], but interpretation often falters at a central gap: nominal dosing rarely reflects the concentrations actually attained in the human body. Without exposure context, dose–response curves are difficult to compare across devices and laboratories, and *in vitro–in vivo* translation (IVIVT) remains uncertain. ML-based pipelines have advanced robust, high-throughput readouts—for example, automated imaging to detect cisplatin nephrotoxicity in patient-specific kidney organoids [96] and ML-assisted high-content analysis to predict neurotoxicity in midbrain organoids [97]. Yet, even precise phenotyping does not reveal what exposure the tissue experienced. This motivates explicit concentration modeling so that observed effects can be anchored to cellular drug levels. Physiologically based pharmacokinetic (PBPK) concepts adapted to the *in vitro* setting, and linked to patient-scale PBPK/pharmacodynamics (PD), offer that anchor. A recent tailored mechanistic framework illustrates the approach: an ordinary differential equation (ODE) 'digital twin' partitions compound between medium, interstitium, and intracellular space while accounting for biological, hardware, and physicochemical determinants (binding, permeability, partitioning, and device adsorption), thereby separating passive transport from active metabolism [98]. Such models enable (i) back calculation of intracellular exposure underlying toxicity phenotypes, (ii) principled comparison across culture formats (ECM domes, microfluidic chips, and bioreactors), and (iii) forward projection to patient PK/PD for prospective IVIVT.

Organoids provide physiologically relevant systems that facilitate drug discovery, from early-stage disease modeling through target and candidate identification, toxicology testing, and personalized medicine. AI- and ML-based computational approaches are increasingly integral to these developments, and digital twin technologies promise to further revolutionize toxicology evaluation and PBPK modeling.

Coculture approaches

Traditional organoid models often lack the native tissue microenvironment, including vasculature, immune cells, microbes, and other stromal components, thereby limiting physiological relevance. Tremendous advancements have been made in immune cell cocultures, allowing systematic investigation of immune infiltration, activation, and cytotoxicity in a 3D context. For instance, colorectal cancer organoids with autologous immune cells modeled responses to immune checkpoint inhibitors, uncovering resistance markers such as the REG4 signature [99]. Nevertheless, immune cells show poor infiltration and interaction with bulk organoids in ECM domes, limiting robust quantitative readouts. Automated ML platforms, such as Organoid App [55] and OrganoIDNet [56], accurately distinguish organoids from immune cells, debris, and imaging artifacts and have been successfully applied to monitor organoid coculture responses to chemotherapeutic and immunotherapeutic treatments [56] (Table 1). Liu *et al.* [100] incorporated AI

(YOLO neural network) to advance distribution accuracy of single-organoid lung cancer PDOs and T cells via microneedle robotics on a microwell chip. By detecting each organoid in real time, an AI-based method guided the microneedle to avoid empty wells or multiple organoids per well, thereby improving distribution accuracy. This approach could link scRNA-seq with anti-PD-1 responses and identify novel regulators of T-cell activity. Ultimately, ML/AI approaches could guide immune digital twin generation, a hypothesized computational model that could potentially integrate patient-specific tumor and immune data to simulate tumor–immune interactions and predict individual responses to therapy [101]. Immune cells and epithelial cells are influenced by neighboring stromal fibroblasts. In particular, in the context of the tumor microenvironment, cancer-associated fibroblasts (CAFs) critically influence the patient's clinical outcome by secreting growth factors, inflammatory ligands, and ECM proteins, which drive tumor progression, hinder immune cell infiltration, and alter chemoresistance [102]. Xu *et al.* [103] developed a LASSO-refined CellChat-based prognostic index for CAF-cancer cell crosstalk-related gene pairs identified via scRNA-seq that predicted immunotherapy response of patients with head and neck squamous cell carcinoma. Although the use of ML/AI methods for the analysis of organoid coculture systems is still in its early stages, it represents a crucial step toward extracting relevant information for future biological platforms that enhance physiological accuracy and relevance.

However, accurate vessel segmentation is already possible in sparsely labeled histology samples of human pancreatic tumors using U-Net neural network approaches and lays an important foundation for organoid models [104]. Ahmad *et al.* [105] introduced a label-free approach to analyze the dynamics of leukocyte trafficking in a vasculature-on-a-chip model. Their pipeline integrates a semantic segmentation algorithm and a classification script using a tracking algorithm, which links nearest neighbors and can cope with the changing phenotype, localization, and time during leukocyte transmigration through the endothelium monolayer. ML is also implemented in the approaches of Tronolone *et al.* [106] to correlate the morphology and ML-determined quality of vasculature networks to oxygen transport efficiency. Predicting the oxygen transport efficiency of vasculature before establishing organoid coculture would enable the efficient generation of more robust vascularized models in the future.

Lack of vascularization causes hypoxia, nutrient deprivation, and necrotic cores; in 3D-printed breast cancer organoids, vascularization increased proliferation and migration relative to nonvascularized controls [107]. Vessels also modulate drug delivery: nearly 97% of administered nanoparticles enter tumors via active transendothelial transport, and ML-based segmentation revealed substantial heterogeneity in vascular permeability and nanoparticle uptake across tumors—an opportunity for personalization [108]. Technical hurdles include vessel segmentation in endothelial–organoid cocultures; nonetheless, a U-Net-style neural network yields accurate vessel maps even in sparsely labeled pancreatic tumor histology, providing a foundation for vascularized organoid analysis [104]. Label-free pipelines now quantify leukocyte trafficking in vasculature-on-a-chip by coupling semantic segmentation with nearest-neighbor tracking [105], and ML metrics of network morphology correlate with oxygen transport efficiency [106], enabling *a priori* assessment of perfusion quality before coculture setup.

Microbiota also shape organ function, especially in gut models; dysbiosis is associated with combined immunodeficiency, autoimmune disease, and IBD [109]. Intestinal organoid–bacteria cocultures have been established [110], and ML improves the detection of mutational signatures from toxin-producing pks^{+} *Escherichia coli*: increased sensitivity revealed a bacterial signature in 12% of patients with colorectal cancer [111,112]. Looking ahead, ML/AI should help identify beneficial microbial consortia that promote maturation and predict culture conditions for stable organoid–microbe coexistence.

Together, advances in immune, vascular, stromal, and microbial cocultures—amplified by ML/AI—are bringing organoids closer to native tissue complexity while opening routes to standardized, quantitative, and clinically relevant readouts.

Environmental control and emerging platforms

Stem cells and organoids derived from them are sensitive to their extracellular environment. Factors that influence morphogenesis, cell fate, and function of the cellular ensembles include the stiffness of the surrounding, shear stress, and mechanical forces. However, measuring mechanical forces in complex biological settings is challenging. To estimate intercellular mechanical forces, Liu *et al.* [100] developed MATCHY (Multiplexed Adhesion and Traction of Cells at High Yield), a semiautomated agent-based ML framework that integrates microfabrication-based traction force and cell–cell adhesion assays with immunofluorescence-based cell identification, enabling high-throughput analysis of the mechanical properties of complex cellular mixtures such as embryonic kidney tissue. MATCHY could be retrained on organoid data in the future. However, this approach focuses on single cells and neglects spatial context. Rozman *et al.* [113] developed a surface tension-based vertex model of a single cell layer-thick epithelium as a simplified organoid-like model that predicted many observed organoid morphologies and can be used to study collective mechanical effects in 3D tissue culture models. Yang *et al.* [114] demonstrated that organoid crypt formation in intestinal organoids is an orchestrated mechanism between lumen volume reduction, actomyosin-mediated contraction of the crypt-resident cells on the apical side, and simultaneous basal tension in villus-located cells. Based on 4D multiscale imaging, scRNA-seq, and perturbation studies of a biophysical model (3D vertex model), they uncovered that cell differentiation initiates these changes and ultimately induces crypt formation. By combining longitudinal live-cell imaging and scRNA-seq of brain organoids with computational integration approaches, Jain *et al.* [115] identified the importance of the ECM as a key regulator of morphodynamics and cell behavior during brain development. Aberrant regulation of extracellular stiffness plays a critical role in brain malformation, as demonstrated by Karlinski Zur *et al.* [116] in a brain organoid model of lissencephaly caused by the LIS1 mutation. Using a mechanistic model, the authors linked mechanical alterations to ECM reorganization and proposed potential therapeutic strategies to counteract viscoelastic abnormalities. Balbi *et al.* [117] employed a continuum model to disentangle the respective contributions of the inner core contraction and the outer cortical microstructural remodeling to the overall morphogenesis of cortical organoids. More research is being conducted on the biofabrication of complex models that take environmental forces into account. Drakoulas *et al.* [118] developed an ML-based framework to predict the best suitable design of scaffolds for bone regeneration. They constructed probabilistic reduced-order ML model based on high-fidelity simulations of bending and perfusion flow to efficiently predict surface shear strain and wall shear stress at the scaffold surface and used these predictions for sensitivity analysis and multiobjective optimization of scaffold designs. Even though this model is generated for bone biofabrication, it incorporates many aspects that are crucial for the influence of the properties of the biomaterial, flow rates, and shear stress and lays an important foundation for the prediction of different tissue organoid models. New biomaterials are being developed to reduce animal-derived components, improve reproducibility, and maintain optimal drug-binding and gas permeability. AI-driven strategies in biomaterial optimization for organoid culture have been reviewed in depth by Bai *et al*. [119].

Oxygen and nutrient availability are critical determinants of organoid growth, cellular composition, development, homeostasis, and functional maturation [120]. Conventional hydrogel domes of the ECM provide a straightforward method for organoid culture; however, as organoids expand and their metabolic demands increase, restricted nutrient and oxygen diffusion into the inner scaffold

often results in necrotic core formation and reduced cell viability [94,121]. Other advanced organoid culture systems that enable more efficient oxygen and nutrient supply include vascularization of organoids (as discussed in the previous section), microfluidic platforms such as organoid-on-a-chip technologies, and bioreactors [122,123]. Bioreactors offer an alternative culturing strategy that ensures homogeneous nutrient and oxygen distribution, enabling large-scale production of organoids with improved uniformity and enhanced data robustness for downstream analyses. To determine the optimal operating regime of bioreactors with regard to organoid number and reproducibility, Ellis *et al.* [124] developed a continuum mathematical model that incorporated cell seeding density and inlet flow rate to quantify nutrient and metabolite distributions within the bioreactor and included organoid-specific characteristics such as proliferation rate and glucose consumption to guide bioreactor operation. A mesofluidic bioreactor, designed using reaction–diffusion scaling principles, facilitates efficient nutrient delivery and long-term organoid culture. When combined with ML-based classification of longitudinal imaging data, this platform enables noninvasive assessment of organoid development and quality, providing a versatile framework for establishing reproducible cultures [125]. In addition, a tubeless fluidic circuit board with reversibly sealed culture bricks has been developed for dynamic culture of ESC-derived thyroid follicles, enabling flexible plug-and-play configurations via LEGO-like interconnectors [126]. Complementary strategies exploit micromanipulation platforms to spatially constrain cells, supporting patterned and complex biofabrication of organoids [127]. Chip technologies are not only relevant for improved nutrient supply but also offer a highly relevant platform for studying the impact of shear stress on different biological aspects. Shear stress has diverse impacts on organoids, including development, cell diversity, differentiation, and especially reproducible quality in human cerebral organoids, retinal organoids, and complex liver-on-a-chip models [128–130]. Integration of ML for generation, analysis, or modeling of iPSC- or adult stem cell-derived organoids-on-a-chip has rarely been performed. Future efforts should aim to develop organoid culture platforms capable of simultaneously modulating stiffness, pH, and flow dynamics to systematically investigate environmental influences on cellular behavior. Finally, automation and robotics are increasingly used to standardize data acquisition, reduce variability, and enable high-throughput analysis of organoid cultures [131]. These developments collectively provide a comprehensive experimental foundation to inform and validate mathematical models of organoid systems.

Transplantation

Organoid transplantation has emerged as a versatile platform for regenerative medicine, disease modeling, and tissue repair. For example, human pluripotent stem cell-derived cortical organoids integrate into host circuits after transplantation and establish functional connectivity [132]. A recent study in NOD/SCID mice tracked transplanted iPSC-derived islet organoids using 3D magnetic particle imaging. Quantitative, longitudinal assessment of the grafts was achieved with an unsupervised k-means+ clustering algorithm applied to the 3D MPI data, enabling automated segmentation and estimation of the total iron value over time [133]. ML algorithms that were developed to assess proliferation rates, differentiation efficiency, and specific gene expression profiles based on imaging data could be used in the future to select the most promising organoids for successful engraftment [134]. The integration of ML into xenograft experiments has the potential to substantially enhance experimental efficiency while enabling advanced anatomical visualization and precise graft tracking through positron emission tomography and magnetic resonance imaging [135]. Aside from cell or organoid applications, ML algorithm techniques are applied to optimize donor–recipient matching [136], allow virtual biopsy for judging donor organ quality [137], and forecast posttransplant outcomes [138]. Collectively, these approaches highlight how combining advanced imaging and ML can enhance both experimental and clinical transplantation outcomes.

Computational approaches: ML/AI and mechanistic mathematical modeling

Our assessment of recent work at the interface of organoids and computational sciences—as outlined in the previous sections—highlights two complementary classes of computational approaches: ML and AI and mechanistic mathematical models.

ML and AI methods learn patterns from high-dimensional data and are the default choice when organoid datasets are high-dimensional and heterogeneous (microscopy images and videos, sc, and spatial omics) (Figure 2A). They power robust segmentation and 3D reconstruction, time-lapse tracking, lumen/bud detection, and morphology embeddings for screening

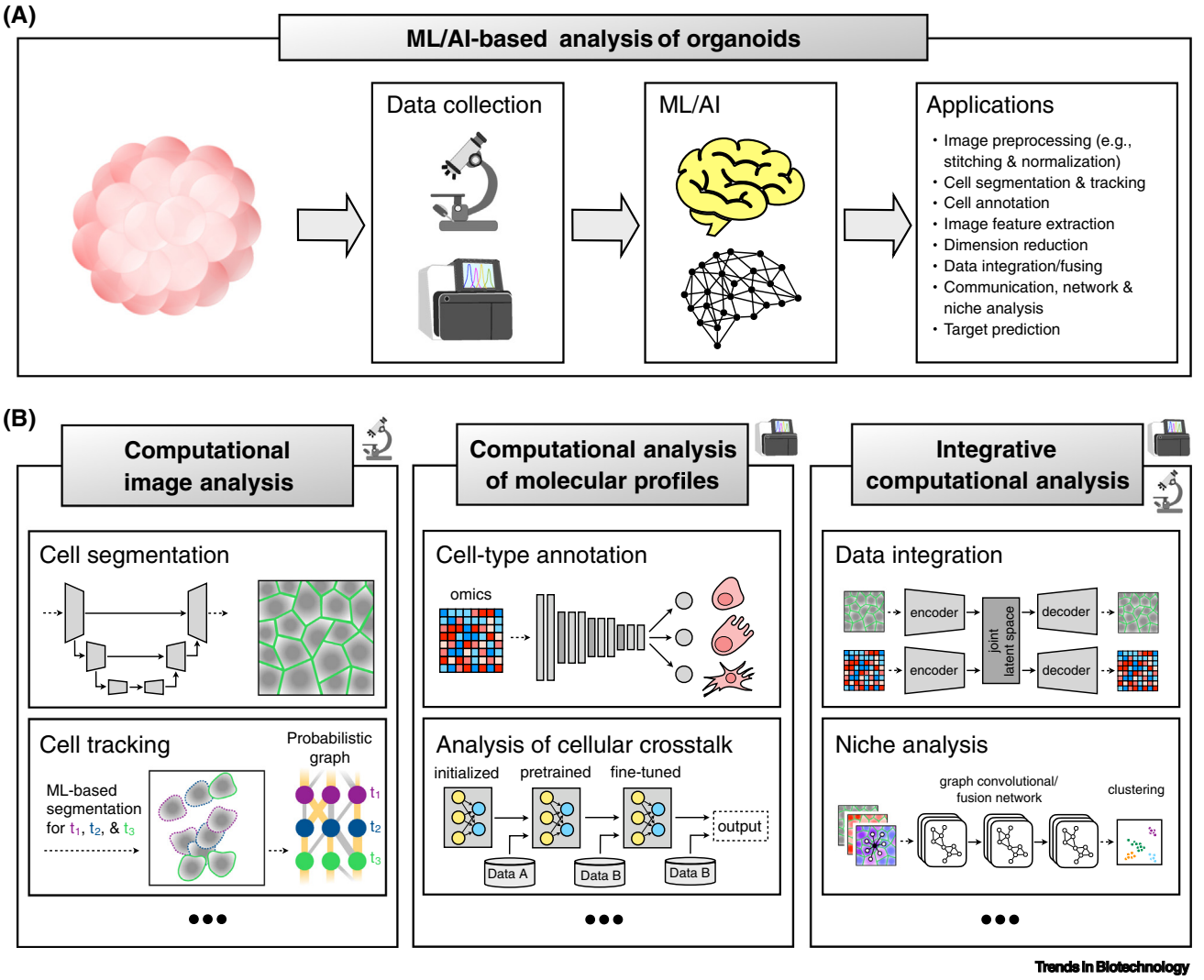


Figure 2. ML/AI applications in organoids. (A) ML/AI-based organoid analysis pipelines involve data acquisition (microscopy and sequencing), ML/AI-based analysis (e.g., neural networks and graph-based models), and downstream applications. (B) Major application domains include data-specific analyses such as image analysis, analysis of molecular profiles, and integrative analysis. (Left panel) ML-based cell segmentation and tracking from microscopy images are illustrated, including the use of neural networks for cell segmentation and probabilistic graph models to link segmented cells across multiple time points (\(t_1\), \(t_2\), \(t_3\)). (Middle panel) Molecular measurements (e.g., transcriptomic or proteomic data) are used for cell-type annotation and for analysis of cellular crosstalk, including transfer learning approaches in which pretrained models are fine-tuned on organoid-specific data. (Right panel) Multiple data modalities are combined through data integration architectures (e.g., encoder–decoder frameworks) to enable joint analysis, optionally followed by higher-level analyses such as niche analysis using graph-based or fusion network models and downstream clustering. AI: artificial intelligence; ML: machine learning.

(Table 1). Beyond vision, they integrate imaging with (spatial) multiomics by learning **joint latent spaces** to build atlases, predict cell states and cellular niches, and connect gene expression to structure and function. The structure of the neural networks depends on the specific task, ranging

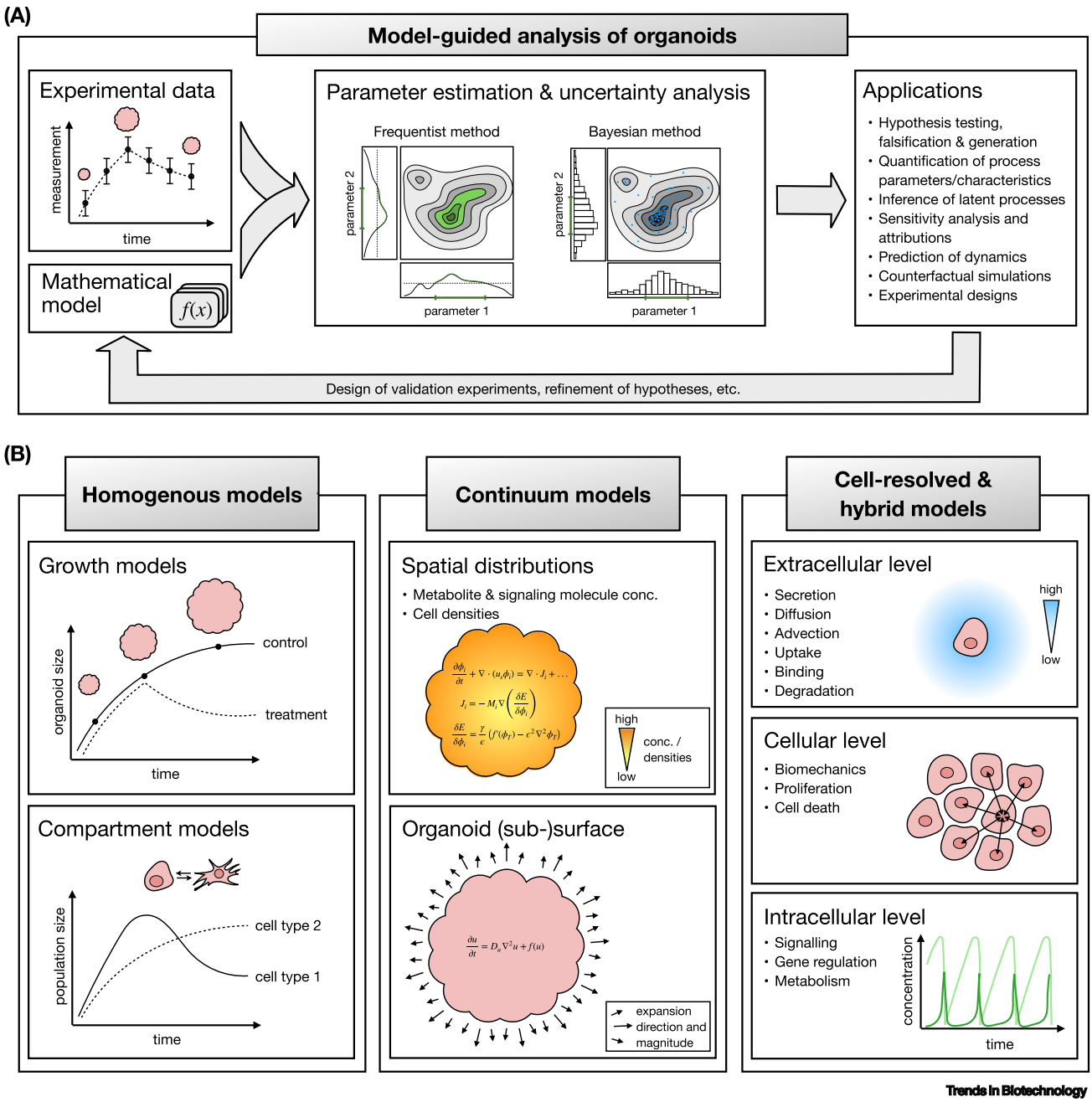


Figure 3. Mechanistic modeling for organoids. (A) Model development is an iterative process where experimental data are used to calibrate models (parameter estimation and uncertainty quantification). Calibrated models can then be used in their application of interest, for example, to design validation experiments or for **counterfactual simulation**. (B) A variety of models are available that are suitable for different applications. Homogeneous models (left panel) can describe growth (growth models) or cell population dynamics via interacting compartments (compartment models). Continuum models (middle panel) incorporate spatial aspects to describe molecular concentration and cell density distributions and to impose restrictions at the organoid surface or subsurfaces. Cell-resolved and hybrid models (right panel) are suitable for cell-specific interactions on the extracellular or cellular level, while supporting a detailed description on intracellular level. conc: concentration.

from feed-forward networks to (graph) CNNs (including U-Net-like networks), autoencoders, and (transformer-based) large language models (Figure 2B). Standard training–validation test setups are used, as well as pretraining in combination with refinement. Because performance critically depends on the observables, their spatiotemporal/molecular resolution, and their **batch/noise structure** (shaped by matrix vs. scaffold-free culture, microfluidics, cocultures, and automation), rigorous crossvalidation, and uncertainty calibration are essential. Recently, ML/AI methods have also been embedded in robotic systems to facilitate automated sample handling [86,131].

Mechanistic mathematical models encode prior knowledge to, for example, assess and generate hypotheses, quantify process parameters, and predict functional characteristics under perturbations (Figure 3A). There is a broad range of model formalisms [139,140] that are used depending on the biological question and the data availability, and this range can be roughly categorized into three classes (Figure 3B). **Homogeneous models**, such as ODE models capture organoid-level dynamics (e.g., logistic/Gompertz growth and exposure–effect couplings) and scale to cohorts, quantifying heterogeneity and dormancy. Continuum partial differential equation (PDE) models describe spatial transport and patterning (nutrient/oxygen gradients, moving boundaries, and **mechanochemical instabilities**). **Cell-resolved models** and **hybrid models** (agent-based models, vertex models, and cellular Potts models, with PDE fields and intracellular ODEs) reproduce budding, branching, lumenization, and fusion. Parameters are inferred via multistart optimization with profile likelihoods (for **identifiability**/confidence) or Bayesian schemes (MCMC/ABC) for stochastic/hybrid cases; model comparison and posterior predictive checks guard against mismatch.

Mechanistic mathematical models today are typically calibrated on quantitative datasets that have been preprocessed and quantified with ML/AI pipelines. In this sense, mechanistic modeling directly depends on ML/AI to transform raw measurements into model-ready observables. Conversely, ideas from mechanistic modeling increasingly inform ML: interpretable and causally oriented approaches help connect predictors to mechanisms, while physics-informed ML/AI and surrogate models embed scientific constraints or emulate simulators to accelerate design and inference [141].

Concluding remarks and future perspectives

Organoids have emerged as a powerful model system, bridging clinical research, animal research, and traditional cell culture. While the development of more complex and physiologically relevant organoid models is on the rise, subsequent data analysis remains challenging. The integration of organoids with computational analysis and mathematical modeling offers unprecedented opportunities to dissect complex biology, predict experimental outcomes, and accelerate translational impact. Yet, variability in experimental systems, the demand for multimodal data integration, and constraints in privacy-preserving data sharing remain critical hurdles. Moreover, limited data availability increases the risk of model overfitting, leading to computational models that are biased toward the patient populations represented in the training data. Further uncertainty arises when models provide predictions without transparent reasoning. In the absence of integrated biological background knowledge, the model interpretability should therefore be rigorously validated against an independent reference dataset (see Outstanding questions and Figure 4).

Looking ahead, AI-guided computational frameworks and hybrid modeling approaches will be central to overcoming these barriers, reducing experimental inconsistencies, and enabling the convergence of molecular, imaging, and patient data across spatial and temporal scales.

Outstanding questions

How can we overcome experiment costs, donor material access, and specific disease (stage) biases to ensure broad data availability, to reliably train computational models, and to reduce the risk of overfitting in the future?

How can artificial intelligence be implemented to reduce laboratory-to-laboratory variability in culture conditions and experimental setups?

How will we manage data processing to cope with large data files while increasing throughput, experimental duration, integrating high and low dimensions, and introducing complex coculture organoid models?

How will we achieve real-time updating of models with new patient data while preserving patient privacy?

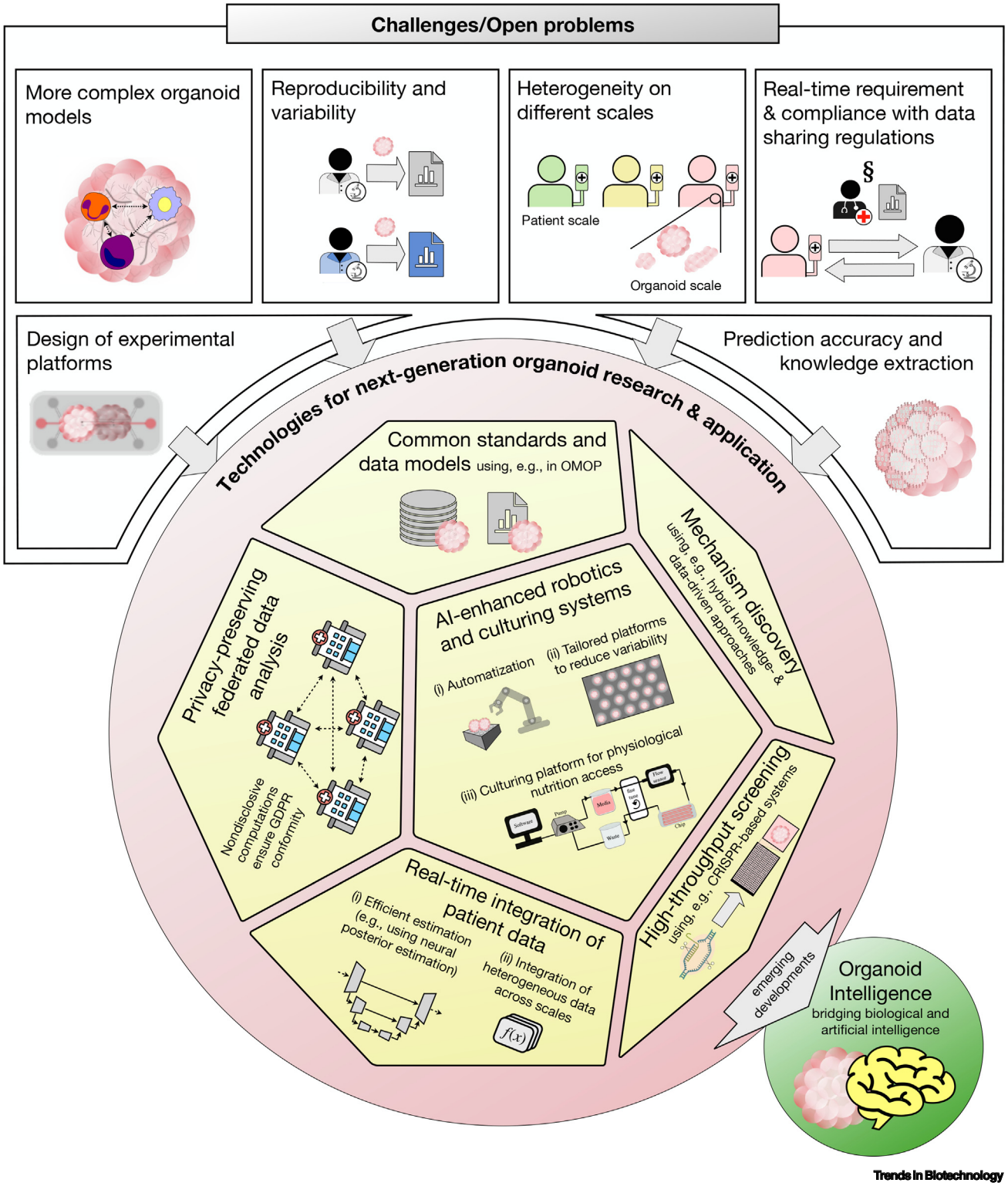
How do we ensure patient protection when mathematical models support organoid-guided therapy?

Who among the patient, developer, and commercial entities owns the organoids and controls their usage?

Should clinical translation be accelerated through, for example, conditional early approvals and risking premature clinical use? Limited *in vivo* human data and an uncertain risk–benefit balance pose significant ethical hurdles for first-in-human trials.

Is the Human Fertilization and Embryology Act 1990, which restrictsresearch with embryo models and embryoids to 14 days after fertilization, outdated? Ethical debates about the onset of life and consciousness question the use of embryonic stem cells for embryo and brain organoid generation and might prompt further restrictions on working with these models.

How do we ensure that artificial intelligence/machine learning decisions are biologically relevant rather than black boxes? How much prior biological knowledge is necessary to ensure relevant data interpretation by artificial intelligence/machine learning? Can benchmark datasets be implemented for model validation?



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Federated learning approaches can also protect patient data, while additional experimental standardization facilitates the clinical implementation of organoid-based research. At the same time, evolving ethical and legal frameworks are necessary to address questions of consent, data ownership, and the responsible clinical translation of organoid technologies.

Ultimately, the convergence of organoid biology and computational modeling holds the potential to redefine biomedical research and therapeutic development—enabling a new era of discoveries. Indeed, even an integration of biological and artificial intelligence, yielding brain-inspired AI hardware [142], might be possible.

Author contributions

Conceptualization: J.H. and E.S.R.; literature research: S.P.R., J.B.P., D.P., S.M., C.M., J.H., and E.S.R.; visualization: S.P.R., J.B.P., S.M., J.H., and E.S.R.; writing—original draft: S.P.R., J.B.P., D.P., S.M., C.M., J.H., and E.S.R.; writing—review and editing: S.P.R., J.B.P., D.P., S.M., C.M., J.H., and E.S.R.; funding acquisition: D.P., C.M., J.H., and E.S.R.; supervision: J.H. and E.S.R.

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Declaration of interests

C.M. is on the board of the European Organ-on-Chip Society (EUROoCS).

Resources

[i]https://www.fda.gov/news-events/press-announcements/fda-announces-plan-phase-out-animal-testing-requirement-monoclonal-antibodies-and-other-drugs

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Figure 4. Challenges and perspectives for next-generation organoid research. Overview of current challenges (top) and enabling technologies (bottom) in organoid research. Key challenges include increasing biological complexity, reproducibility and variability, heterogeneity across patient and organoid scales, real-time data processing requirements, compliance with data sharing and privacy regulations, design of experimental platforms, and prediction accuracy and knowledge extraction. Enabling technologies include standardized data representations and common data models, privacy-preserving and federated data analysis, AI-enhanced robotic culturing systems to reduce variability, real-time integration of patient and organoid data via efficient parameter estimation methods such as neural posterior estimation, where neural networks transform simulations into posterior parameter distributions, and integration of heterogenous data into joint latent spaces, high-throughput screening platforms, and computational approaches such as hybrids between knowledge- and data-driven methods for discovering mechanisms. Together, these advances support the development of organoid intelligence, integrating biological and AI for scalable and reproducible applications. AI: artificial intelligence.

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