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Organoid Array Computing
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Organoid Array Computing
The Design Space of Organoid Intelligence

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Abstract

In this paper, we explore the artificialization and networking of biological matter via brain organoids—three-dimensional, stem-cell-derived structures that recapitulate aspects of human brain architecture and function. These organoids serve as a platform for investigating the emergent properties of biological neural networks and the potential for developing an in-vitro to in-silico cognitive architecture. Our research addresses the burgeoning field of organoid intelligence (OI), wherein biological substrates are interfaced with computational systems, providing an adaptive framework for embodied computation. A common distinction between software and hardware in the field of biocomputing assumes DNA as software and cells as hardware. By evolving through biochemical and physical signaling feedback, organoids challenge this dichotomy. OI integrates both, enabling biological systems to move along the continuum from software to hardware into a multiscale machine. We begin by examining the current interfacing technologies that enable the connection between organoids and digital systems, evaluating the proof-of-concept studies that have laid the groundwork for OI applications. This analysis includes a critical assessment of the existing practical and technical limitations that hinder the realization of scalable OI. We then propose design strategies aimed at overcoming these obstacles, emphasizing the need for a nested approach to experimental design. New permutations enable the iterative development of OI modules, facilitating the integration and application of polycomputational neural assemblies. The design space of OI focuses on the growing dimensions and analysis of inputs, outputs, interfaces, and frameworks across multiple scales. We posit that the design of OI is less an act of top-down design and more a process of guided evolution, wherein higher-order cognitive functions emerge organically from the intricate interplay of lower-level biochemical substrates. Through this, we speculate on how higher-order functions can emerge from networking biological matter from embedded substrates “downstream”. Our research aims to uncover new dimensions in the information-processing capabilities of OI, positioning OI as a novel form of AI.

Keywords

artificial intelligence; human brain organoids (HBOs); organoid intelligence (IO); interface; biological neural network; hybridized entities; biocomputing

1 Introduction

The discovery of new materials and functional substrates continually reshapes novel approaches in computing and provides "new means of acting on and interpreting the world."[1] Recent advancements in stem cell research have opened groundbreaking avenues for utilizing human neuronal tissue as a substrate for unconventional neural networks. At the forefront of this research are brain organoids: complex structures derived from stem cells that develop functional neural networks, enabling the artificialization of biological matter and the networking of growing and thinking matter.[2]

Organoids, when interfaced with artificial intelligence through multielectrode arrays (MEAs), present a compelling framework for instantiating novel modes of computing and neural network architectures. This paper explores the design space of organoid intelligence (OI), traversing the continuum from organoids as "software" emerging from lower-level biological substrates to their role as "hardware" supporting higher-order computational processes.

If the word *computer* refers to any physical object that can implement any computable function, then biological brains—and, by extension, human brain organoids (HBOs)—are literally computers.[3] Viewing biological neural tissues as computational entities enables us to move beyond the conception of computers as externalized brains, instead considering artificialized brain models as computers themselves. Further, as current developments in artificial intelligence point toward a "hardware bottleneck issue," this position enables us to depart from neuromorphic designs in silicon and to examine the inherent computational power of living neuronal tissues as a potential solution.[4]

We propose viewing the design space of OI as a "polycomputational" neural assembly, integrating hardware, software, organoids, and chemical substrates into a cohesive computational framework that couples in-vitro biological systems with in-silico computational frameworks. In the following sections, the paper will elucidate and expand on the design space of OI, delineating the various dimensions crucial to experiment design in this emerging field. We will explore the building blocks of OI experiments, considering inputs, outputs, interfaces, and frameworks across multiple scales. By mapping this design space, we seek to provide a comprehensive foundation for future OI experimentation and its potential applications in artificial intelligence.

2 Background on Organoids

Organoids are 3D tissue cultures derived from stem cells that model the structures and functions of an organ.[5] The discovery of organoids began with Hans Clevers's groundbreaking work, in which organoids were first grown in lab petri dishes using stem cells from patients' small intestines.[6]

Advancements in stem cell research and tissue cultures have blurred the lines between biological and artificial systems. Key developments include the creation of chimeric embryos, which combine genetic material from different organisms, and synthetic tissue cultures that challenge the primacy of DNA in determining biological outcomes.[7] These breakthroughs not only expand our understanding of biological plasticity but also pave the way for innovative research in regenerative medicine, organ transplantation, and the study of human evolution and neurodevelopment.[8]

Today, stem cell cultures can be programmed and exposed to specific environmental factors to functionally model various organ sections.[9] HBOs, also referred to as cerebral organoids, are grown from human pluripotent stem cells that can mimic aspects of the architecture and functionality of the human brain (see Figure 1).[10] HBOs are traditionally utilized for studying human brain development, modeling neurological diseases, testing drug efficacy, and other assessments of neurodevelopmental processes that would otherwise present ethical and practical constraints associated with human brain research.[11]

Although HBOs have the ability to replicate certain aspects of brain structure and function, they are currently limited in their scale and complexity. Without solving significant gaps in vascularization and interorganoid communication, organoids remain a minimum "working model of some of the circuitry resident in a living, functioning human brain."[12]

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[1] Beaulieu et al., "Refractive Computation,"
[2] Smirnova et al., "Organoid Intelligence: New Frontier"
[3] Richards and Lillicrap, "Brain-Computer Metaphor."
[4] Mencattini, "Assembloid Learning."
[5] Smirnova et al., "Organoid Intelligence: New Frontier"
[6] Sato et al., "Single Lgr5 Stem Cells."
[7] Blakemore, "Human-Pig Hybrid"; Kruszelnicki, "Mouse with Human Ear"; Tissue Culture & Art Project, "Crude Matter."
[8] (Sun et al., "Applications of Brain Organoids" 2021; Chen et al., "Human Brain Organoids." 2019
[9] Fernandes, "Organoids as Complex (Bio)Systems."
[10] Takahashi and Yamanaka, "Induction of Pluripotent Stem Cells"; Baldassari, "Brain Organoids."
[11] Sun et al., "Applications of Brain Organoids."
[12] Goldman, "Assembloid Models"; Smirnova et al., "Organoid Intelligence: New Frontier."

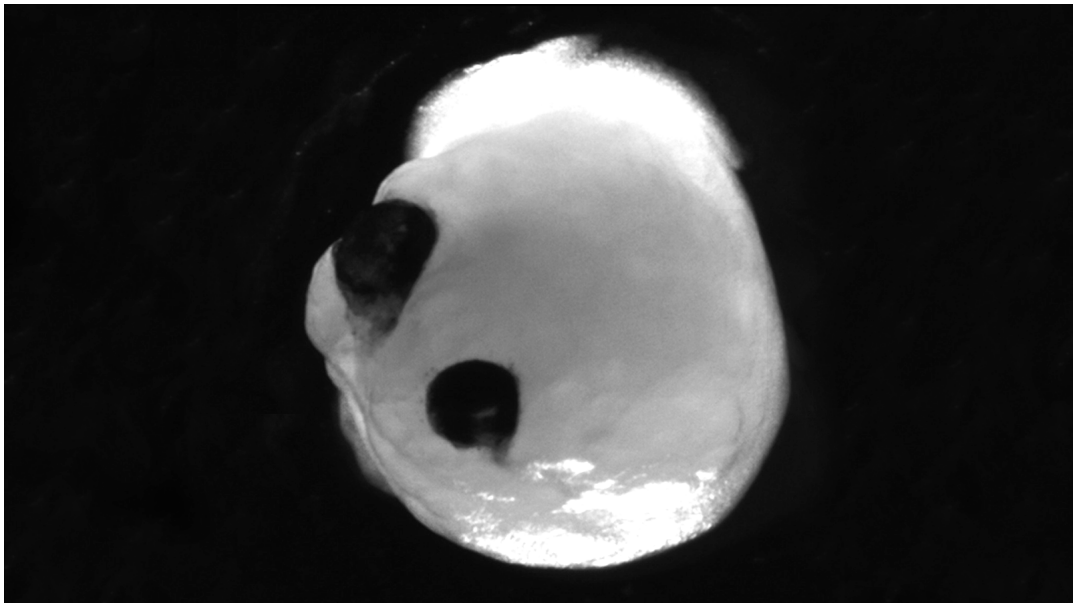

Figure 1 Sixty-day-old organoids with bilaterally symmetric pigmented optic vesicles.[13]

2.1 The Concept of Organoid Intelligence: In-vitro to In-silico Interface

An organoid in a petri dish exists in an in-vitro state, isolated from external inputs and without perceivable outputs. However, HBOs have now been interfaced with artificial intelligence, creating an interconnected system where AI serves as an analytical tool to process high-dimensional data from these biological structures.[14] This is referred to as "organoid intelligence" (OI), a term first introduced in the article "Organoid Intelligence (OI): The New Frontier in Biocomputing and Intelligence-in-a-Dish."[15]

OI is the hybridization of biological computing with machine interface technologies. This integration enables us to virtually embody organoids, transitioning them from an in-vitro to an in-silico "in computer" instance. The physical organoid interface includes three central components: an HBO, an MEA, and a microfluidic platform.[16] Each element plays a crucial role in creating a functional and interactive bio-electronic interface (Figure 2).

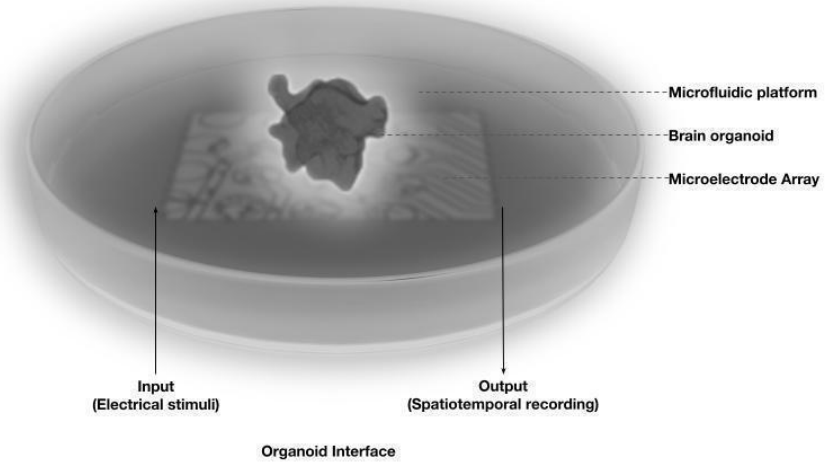

Figure 2 Typical interface for OI. Figure designed by Jenn Leung.

[13] Gabriel et al.. "Human Brain Organoids." 2021
[14] Smirnova et al., "Organoid Intelligence: New Frontier."
[15] Smirnova et al., "Organoid Intelligence: New Frontier." 2023
[16] Smirnova et al., "Organoid Intelligence: New Frontier"

At the core of the tripartite interface, we have HBOs, which serve as functioning neural networks capable of processing information. HBOs' inherent ability to form and reorganize synaptic connections means that they can be trained, opening up new possibilities for research and application.

MEAs are used for interfacing HBOs with external systems, enabling precise delivery of electrical stimuli and recording of neuronal activity.[17] Different types of MEAs, such as shank and mesh electrodes, offer specialized functionalities, with shank electrodes allowing access to deeper layers of organoids and mesh electrodes providing flexible interfaces.[18] This bidirectional communication probes neuronal networks within the organoids and stimulates their development (Figure 3).

In addition to MEAs, the microfluidic platforms that house the organoids are essential to their sustained functionality. Typically housed in a petri dish, microfluidic platforms deliver a carefully balanced culture medium that supports cell growth and development.[19] These platforms simulate the microvascular networks of the human brain, delivering a continuous flow of culture medium that mimics the nutrient and waste exchange found in vivo. This controlled environment ensures that the organoids remain healthy and responsive over extended periods, thus maximizing their utility in experimental setups.[20]

Together, these technologies enable us to process and study organoid output, offering insights into their computational potential and applications in neurological research. In the following sections, we will explore multiple frameworks for OI application, tracing the evolution from neuromorphic computing to direct organ-on-chip systems.

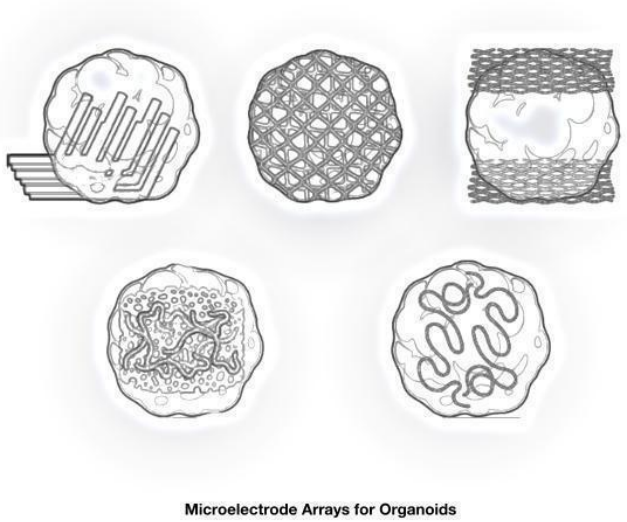

Figure 3 Various types of MEAs for organoids (adapted from Passaro and Stice,"Electrophysiological Analysis").

2.2 Artificial Neural Networks vs. Organoid Neural Networks

The use of OI as a new form of computation comes partly from the fact that, in learning, organoid neural networks (ONNs) are formed. These could be useful in solving a range of downstream tasks, because they solve the problem of traditional artificial neural networks (ANNs) that are inherently static systems, characterized by fixed topologies. Once an ANN is designed and trained, its structural properties—such as the number of layers, neurons, and connections—remain unchanged.

This is not just the case for simple learning paradigms—such as supervised, unsupervised, or reinforcement learning—but also for more complex subparadigms, such as continual learning (otherwise known as lifelong learning[21]), where the model can learn from new information over time, or self-supervised learning, where the model uses the data itself to generate labels.[22] In all of these paradigms, the models are incapable of changing their own architecture.

This rigidity contrasts sharply with the learning in biological neural networks, such as those formed by organoids, which exhibit fluid intelligence and neuroplasticity. Unlike ANNs, the neurons in a brain or brain organoid can form new connections with other neurons, enabling not only continuous learning but also continuous adaptation of the architecture itself. This dynamic quality highlights the potential of OI, where evolving neural topologies could lead to more flexible and adaptive forms of computation.[23]

[17] Passaro and Stice, "Electrophysiological Analysis."
[18] Passaro and Stice, "Electrophysiological Analysis.".
[19] Quintard, "Microfluidic Platform."
[20] Passaro and Stice, "Electrophysiological Analysis"; Sharf et al., "Functional Neuronal Circuitry"; Quintard et al., "Microfluidic Platform"; Ballav et al., "Organoid Intelligence."
[21] Parisi et al., "Continual Lifelong Learning."
[22] Jaiswal et al., "Contrastive Self-Supervised Learning."
[23] Mencattini, "Assembloid Learning."

3 Current Applications of Organoid Intelligence

Currently, researchers are able to monitor and modulate the neural activity, effectively integrating the biological neural networks in HBOs with electronic systems.[24] The organoids are embedded into computational frameworks that can enable them to perform specific tasks. Neural signals from the HBOs can control virtual environments or robotic systems, enabling the study of learning and adaptive behaviors. Algorithms decode these neural patterns and optimize the interactions between organoids and their virtual or physical embodiments.[25]

OI creates a bidirectional communication system between the HBOs and the interfaced AI system through MEAs. OIs are both "plugged in" to these AI chips and connected to the HBOs' neural activity.[26] In this section, we review a (nonexhaustive) list of current OI case studies.

3.1 DishBrain *Pong*

The virtual and robotic embodiment of OI immerses the biological neural network within virtual or robotic environments, which allows them to interact and adapt in simulated worlds.[27] A prominent OI case study is the "DishBrain" device by Kagan and team at Cortical Labs,[28] which demonstrates how HBOs can be integrated into a simulated game environment of *Pong*.[29]

The primary objective of DishBrain was to explore the capabilities of in vitro neural networks to perform goal-oriented tasks when provided with sensory input and feedback. Kagan and his team grew organoids on MEAs,[30] which recorded and stimulated the electrical activity within the neurons and involved a grid of electrodes that delivered electrical stimuli to specific regions of the neural network.[31] DishBrain was virtually embodied into a game environment that simulated the arcade game *Pong*, where a virtual paddle controlled by the biological neural network interacts with a ball that moves back and forth across the screen as a closed-loop feedback system.[32] If the virtual paddle was successful in hitting the virtual ball, a 'positive' feedback signal was sent to the sensory region to reinforce this behavior, and if the paddle missed the ball, a 'negative', less predictable feedback signal was sent. The biological neural network adapted to the feedback from the game environment and improved its performance, demonstrating learning and adaptive behavior. The research also reveals that DishBrain competes with other existing deep reinforcement learning algorithms.[33] By demonstrating that neurons can learn and adapt in goal-directed ways, DishBrain opens new avenues for brain–machine interfaces, neurocomputational models, and biological–artificial hybrid systems.

3.2 Neandoroids

Neuroscientist Alysson Muotri and his team at the University of California, San Diego, pioneered the development of Neanderthal brain organoids by reintroducing the archaic variant of the NOVA1 gene into human stem cells.[34] These organoids exhibit significant differences from human cortical organoids, including longer growth periods, a distinctive popcorn-like shape, and fewer cortical connections. These findings suggest that the neurological structures of Neanderthals may have influenced their cognitive abilities and social behaviors in ways that differ from those of modern humans, and this research offers a novel platform for studying human evolution and neurodevelopment. Further, Muotri and team connected Neanderthal organoids to robotic systems, allowing these brain models to interact with and explore their environment.[35] This fusion of biology with modern technology opens a realm of new possible research for understanding cognition, learning, and adaptation across evolutionary timescales. This work not only enhances our understanding of human brain evolution but also lays the groundwork for future studies that may uncover the genetic basis of human-specific traits and vulnerabilities.

3.3 Speech Recognition Studies

Guo and his research team at Indiana University Bloomington developed a novel hybrid system called Brainoware. In their study, published in *Nature Electronic*, Guo's team conducted a benchmark test to evaluate Brainoware's capabilities in speech recognition.[36] They used 240 audio clips of Japanese vowels, which were converted into electrical signals and processed by the HBOs. These signals were then decoded by an AI tool. Although the system initially showed low accuracy, it improved with training, eventually reaching a 78 percent accuracy rate. While this is lower than that of conventional

[24] Smirnova et al., "Organoid Intelligence: New Frontier."
[25] Kagan et al., "In Vitro Neurons"; Muotri, "Brain Model Technology."
[26] Greenberg, "Birth of Wetware."
[27] Kagan et al., "In Vitro Neurons"; Smirnova et al., "Organoid Intelligence: New Frontier"; Khajehnejad et al., "Biological Neurons."
[28] Kagan et al., "In Vitro Neurons."
[29] Kagan et al., "In Vitro Neurons."
[30] Kagan et al., "In Vitro Neurons."
[31] Khajehnejad et al., "Biological Neurons."
[32] Kagan et al., "In Vitro Neurons."
[33] Khajehnejad et al., "Biological Neurons."
[34] Trujillo et al., "Reintroduction."
[35] Trujillo et al., "Reintroduction."
[36] Cai et al., "Brain Organoid Reservoir."

ANNs, the study is a pioneering demonstration of how organoid-based systems can learn and perform computational tasks, marking a significant step forward in the development of biocomputers.[37]

3.4 Bioprocessors

Companies like FinalSpark and Emulate are already selling OI technologies as advanced biocomputational devices.[38] OI is claimed to enhance our understanding of brain function and create new forms of biocomputers that could surpass the efficiency and capabilities of traditional silicon-based systems.[39] As these platforms continue to evolve, they hold the potential to not only transform scientific research but also expand what is currently possible in computing and artificial intelligence, creating hybridized entities of biology and technology.[40]

3.5 Internet of Organoids

FinalSpark has developed an initiative that allows real-time online monitoring of their biochips, offering a window into the live neuronal activity of organoids.[41] Through their online platform, users can observe the real-time functionality of neurospheres housed within the MEAs. Individual charts displayed on the platform correspond to a single biochip, where the activity of one neurosphere is tracked. The charts provide detailed information on the electrophysiological signals detected by the electrodes in the MEA, with each signal representing the neuronal activity of the organoid in response to various stimuli. This feature represents a significant step toward integrating biological systems with digital platforms. By offering live views of the organoids' activity, FinalSpark enables researchers, students, and the public to directly witness the complexities of neuronal signaling and the potential of OI.

4 The Design Space of Organoid Intelligence

As we move from the established foundation and current applications of OI into the design space, we delve into the speculative and exploratory. This space is used to envision the future of OI, where current technological capabilities intersect with innovative concepts. This enables us to explore the potential trajectories of OI development, understanding where the hybridization of biological systems and technology could lead, without necessarily advocating for any specific evolutionary path. The importance of the design space lies in its ability to offer a creative framework to anticipate future opportunities and challenges. By engaging with the speculative, we can identify and address the current limitations of OI technologies while imagining how these challenges might be overcome.

Currently, OI technology faces several significant limitations. Some of these include the lack of vascularization in organoids, which restricts their growth and complexity. OI also has other scope and scale limitations around potential interorganoid communication and the quality of microfluidic platforms. These limitations set important boundaries on what is currently achievable, yet this design space enables us to speculate on how these limitations might be addressed in the future, through innovations in bioengineering, computational frameworks, and ethical oversight.

The future of OI looks to the design of OI systems as a guided evolution. The potential of OI can be understood through theoretical frameworks such as Friston's "free energy principle." This principle posits that the brain constantly strives to create a predictive model of the world, minimizing the gap between sensory inputs and its predictions.[42] Applied to organoids, this suggests that their development of structures such as ocular cups indicates a "demand" for more complex sensory inputs. This self-driven complexity reveals the growing computational potential of organoids as a material substrate for intelligence.

Within this design space, we introduce the concept of scaffolding as a layered approach to developing OI systems. Scaffolding here refers to the idea that each layer of development builds on the last, creating increasingly sophisticated and capable systems. The layers of scaffolding we explore are highly speculative, extending the boundaries of current technology and envisioning how future advancements could fundamentally change what OI can achieve.

The layers we discuss include Layer 1: Organoid Array Computing, where multiple organoids work in parallel to enhance computational capacity; Layer 2: Multimodality Processing, which imagines specialized organoids designed to process different types of sensory inputs; and Layer 3: Intergenerational Memory, which speculates on organoids' potential to transmit learned behaviors or information across generations. These layers represent possible future directions in the development of OI, providing a roadmap for how these biological systems could evolve in complexity and functionality.

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[37] Tsanni, "Human Brain Cells."
[38] FinalSpark, "Neuroplatform"; Emulate, "Brain-Chip."
[39] Smirnova et al., "Organoid Intelligence: New Frontier."
[40] Smirnova et al., "Organoid Intelligence: New Frontier"; Smirnova et al., "Organoid Intelligence: Ultimate Functionality."
[41] FinalSpark, "Neuroplatform."
[42] Friston, "Free-Energy Principle."

4.1 Current Limitations: Vascularization

Current organoid technologies face significant limitations due to HBOs' lack of vascularization. While HBOs offer a promising avenue for studying human brain development and disease, they often lack the microenvironment and vascular support necessary for sustained growth and functionality.[43] This deficiency results in necrotic centers due to insufficient oxygen and nutrient supply, limiting their size and complexity. Although efforts to integrate vascular structures into organoids through cocultures with vascular cells or tissue engineering have shown some promise, they have not yet achieved the authentic blood microenvironment required for proper development.[44]

An alternative approach involves the engraftment of HBOs into animal hosts, such as mice, where they can develop functional vasculature and integrate with the host brain's neuronal circuits.[45] This method has shown success in creating mature and functional human brain tissues in vivo, responding to physiological stimuli and demonstrating functional synaptic connectivity. Preliminary indications are that the human neuronal tissue can not only be grafted into rodent neuronal tissue but also receives sensory input and becomes permeated by blood vessels supplying oxygen and nutrients and carting away metabolic waste.[46] However, the use of chimeras raises ethical concerns, particularly regarding the potential for these organoids to develop morally relevant qualities.[47] As the field advances, it is crucial to address these ethical issues proactively to ensure the responsible development and application of OI technologies.

Another potential solution for the significant challenge of vascularization is bioprinting. While it is commonly associated with creating skin grafts or ear transplants, recent research has employed this technique for introducing vascular structures into organoids.[48] The development of bioprinting has traversed several stages, from first bioprinting nonbiocompatible structures, to nonbiodegradable prostheses, and toward biocompatible and biodegradable structures that support tissue repair and regeneration.[49] Bioprinting uses different bioinks, such as hydrogel composed of cellulose or collagen. When cells are introduced to the microlattice scaffold, they can grow over this scaffold to turn into a 3D structure so that the bioprinted structure provides physical instructions for growth. At times, these temporary and thermoreversible supports can also be washed away, facilitating plastic development. Currently, researchers are able to print biomimic 3D structures with living cells akin to cellular printing, covering multiple materials and cell types.[50]

4.2 Microfluidic Platforms

Beyond addressing vascularization challenges, we can also reconceptualize microfluidic platforms so that they are conditioned to support responsive, open-ended cell development.[51] To consider the potential computational power of organoid systems beyond pure electrical stimulation, we should reposition microfluidic platforms as an architecture for non-electrical stimulation.[52]

Here, chemical and biological computing systems recursively program each other and exhibit multiscale continuation.[53] A more recent concept proposed by Leroy Cronin, *chemputation*, also points to the increasing interest in metabolic design for polycomputational systems.[54] With this understanding, we can view microfluidic platforms as an additional layer of computation, working in concert with organoid structures and electrical feedback systems.

To optimize microfluidic platforms for OI, we propose to develop systems that allow for the circulation of essential nutrients and growth factors, moving beyond static culture broths. Studies show that an extended culture medium permits the development of mature cell types and cellular diversity.[55] For example, microfluidic platforms contribute to the diversity of inputs that organoids are capable of receiving, as culture conditions and duration are critical factors in neuronal maturation and functionality.[56] Neuronal plasticity can be regulated through different culture media, such as neural induction medium, DMEM, maintenance medium, Neurobasal Medium, and BrainPhys Medium.[57] Not only is neuronal activity measured and conditioned with these media, different media help optimize the exposure of fluorescent compounds to organoids and modulate downstream neuronal networks[58].

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[43] Zhang et al., "Vascularized Organoids."
[44] Mansour et al., "In Vivo Model"; Chen et al., "Human Brain Organoids."
[45] Mansour et al., "In Vivo Model."
[46] Goldman, "Assembloid Models."
[47] Hyun et al., "Ethical Issues."
[48] Kengla et al., "Bioprinting of Organoids"; Wang et al., "Application of Bioprinting"; Ren et al., "Developments and Opportunities."
[49] Wang et al., "Application of Bioprinting."
[50] Wang et al., "Application of Bioprinting"; Skylar-Scott et al., "Orthogonal Differentiation."
[51] Quadrato et al., "Cell Diversity"; Cogoni et al., "ISiCell."
[52] Smirnova et al., "Organoid Intelligence: New Frontier"
[53] Bongard and Levin, "Biological Systems."
[54] Cronin, "Chemputer and Chemputation"; Sha, "Metabolic Approach."
[55] Quadrato et al., "Cell Diversity."
[56] Osaki et al., "Complex Activity."
[57] Zabolocki et al., "BrainPhys Neuronal Medium."
[58] Zabolocki et al., "BrainPhys Neuronal Medium" 2020; Osaki et al., "Complex Activity." 2024

The Chemputer proposed by Cronin is a speculative universal chemical synthesis machine that automates the precise control of chemical reactions, using programmable hardware to execute complex synthesis pathways.[59] This polycomputational approach suggests that if liquids can compute, the chemical microenvironments within microfluidic platforms contribute to the overall computational capacity of the system by shaping the conditions under which biological processes occur. Similar to how the Chemputer uses precise control of reagents and conditions to guide chemical reactions and achieve desired outputs, microfluidic platforms in OI systems could dynamically regulate chemical gradients, nutrient delivery, and waste removal. This controlled environment enables the fine-tuning of organoid development and neural activity, effectively guiding the biological processes that underlie computational tasks. In this sense, the liquid environment becomes an integral part of the system's computational framework, enabling the organoids to perform more complex functions by optimizing the conditions for their growth and interaction.

Another branch of organoid engineering research points to hormonal alteration and dopamine stimulation as additional inputs that can influence organoid growth and development.[60] The stimulation of dopaminergic neurons could entrain and modify the activity patterns of neurons in other regions of the assembloid and induce long-lasting morphological changes.[61]

Through these systems, we may also explore a range of substrate materials and configurations to promote open-ended cell development. Further, we might want to design microfluidic platforms that facilitate bidirectional communication between electric signals and chemical culture components. Recursive chemical-to-electrical artificialization enhances the organoids' ability to adapt, self-programming the development of computational capabilities. This fluid architecture is necessary to support the neuroplasticity and adaptability of ONNs.

4.3 Interorganoid Communication: From Organoids to Assembloids

Organoids are often cultured to grow with closely monitored factors to ensure experiment reproducibility and reliability of electrical recording results.[62] However, most experiments are conducted on a specific type of organoid, lacking the ability to mimic fully matured adult brains that have interregional and intercellular interactions.[63] In relation to organoids' potential for heterogeneous development, Sergiu Paşca, director of Stanford's Brain Organogenesis Program, attributes this phenomenon to the brain's inherent capabilities to self-organize "with its own assembly instructions."[64]

An emerging engineering approach is the combination of different region-specific neural organoids into fusion or assemblies to recapitulate the interaction between brain regions. Fused organoids can mimic neural migration, projection, or functional neural circuits between brain regions.[65] Meanwhile, researchers have also started coculturing differently patterned organoids or combining neural organoids with nonneural tissues to model cell migration and connectivity.[66] For example, Shi and team generated vascularized human cortical organoids (vOrganoids) by coculturing human embryonic stem cells or human-induced pluripotent stem cells with human umbilical vein endothelial cells in vitro.[67] There is also an assembly of blood vessels and brain cells and a trio of cerebral cortex, spinal cord, and muscle organoids demonstrating orchestration.[68] Networking assembloids have shown a sign of neuroplasticity through "short-term potentiation," supporting the fluidity of cognitive architectures.[69]

Beyond fused and assembled organoids, HBOs can also communicate with each other by forming connections through axons, the long, thread-like extensions of neurons that transmit electrical signals.[70] Current technology enables researchers to cultivate reciprocal axon bundles between organoids using specialized silicon elastomer microdevices that provide a microchannel to guide the growth of these connections.[71] These connections have been shown to transmit electrical impulses from one organoid to another, demonstrating a form of communication without external molecular instructions. This research reveals that spatial instructions alone (by design of the microdevice) can sufficiently direct organoid development and regeneration (Figure 4). The design of the microdevice, for example, the number of units and dimensions of its channels, and the choice of biocompatible materials provide physical instructions by determining and structuring certain mechanical forces such as compression, pressure, etc.[72]

[59] Cronin, "Chemputer and Chemputation."
[60] Reumann et al., "In Vitro Modeling."
[61] Reumann et al., "In Vitro Modeling."
[62] Chung et al., "Electrophysiological Recording Platforms."
[63] Makrygianni and Chrousos, "From Brain Organoids."
[64] Goldman, "Assembloid Models."
[65] Bagley et al., "Fused Cerebral Organoids" 2017; Suong et al., "Design of Neural Organoids." 2024
[66] Levy and Paşca, "What Have Organoids."
[67] Shi et al., "Vascularized Human Cortical Organoids."
[68] Bagley et al., "Fused Cerebral Organoids" 2017; Birey et al., "Human Forebrain Spheroids." 2017
[69] Osaki et al., "Complex Activity."
[70] Kirihara et al., "Model of a Cerebral Tract."
[71] Kirihara et al., "Model of a Cerebral Tract."
[72] Kirihara et al., "Model of a Cerebral Tract."

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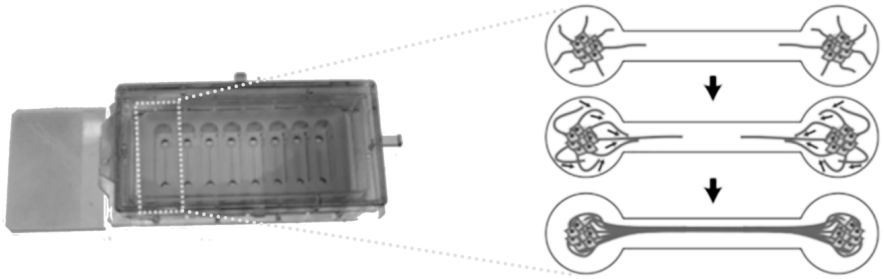

Figure 4 Axon fascicle formation from microdevice physical cues (adapted from Kirihara et al., "Model of a Cerebral Tract.")

When two organoids are connected by an axon bundle, they perform a 'handshake' that leads to synchronized bursts of electrical activity recorded between two organoids.[73] There are existing experiments that support the claim that these interorganoid axonal connections not only correlate to higher short-term plasticity in the neuronal network but also facilitate the development of a higher complexity of signals between connected organoids.[74]

At present, the connections are limited to direct electrical signaling without the intricate synaptic networks found in a fully developed brain. Speculatively, advancing this technology could involve creating more complex microenvironments that promote the formation of more sophisticated neural circuits, including synapses and potentially even chemical signaling pathways. By incorporating factors that encourage the development of these connections, such as growth factors, and using more advanced bioengineering techniques, we could potentially create organoid systems that mimic the complexity of real brain networks and develop robust communication for mutual learning. This could eventually lead to the creation of interconnected organoid networks capable of more advanced, coordinated activities, offering profound insights into brain function and the mechanisms of neurological diseases.

5 Scaffolding for Organoid Intelligence

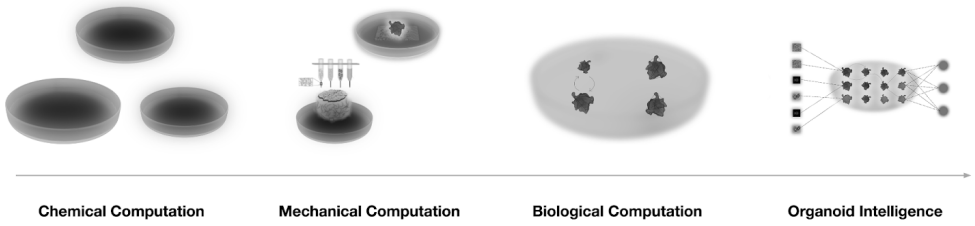

Figure 5 Scaffolding for OI: Chemical computation: designing protocols for culture media and chemical stimuli to influence organoid development, reproducibility, and function; mechanical computation: designing mechanical properties of tissues, biomaterials, and interfaces to provide spatial conditioning and physical cues for organoid growth, e.g. materials, electrophysiological factors, solubility, bioprinting scaffold designs, dimensional parameters, MEAs, and interfacing techniques; biological computation: designing arrays of cellular growth, tissue morphogenesis, maturation, and assembly as well as developing various organoid types; OI: integrates chemical, mechanical, biological, and electrophysiological signal processing to create a comprehensive framework for OI. Figure by Jenn Leung.

Scaffolding for OI relies on a range of dependencies, including microfluidics for nutrient delivery, electric pulses for stimulation, and physical hardware support such as MEAs. However, once these dependencies are in place, HBOs begin to form their own functional neural networks, effectively becoming a form of biological hardware capable of supporting higher-order computational tasks. This continuum of scales—from chemical microenvironments, to mechanical spatial conditioning, tissue

[73] Osaki et al., "Complex Activity."
[74] Osaki et al., "Complex Activity."

scaffolding, and finally, OI—forms the basis of a polycomputational system, where different layers of computation, both biological and artificial, interact to create a complex and adaptable system (Figure 5).[75] Fluid intelligence and neuroplasticity is scaffolding for an OI that has evolving topologies.[76]

To further explore the potential of OI, additional experiments would help refine the encoding and decoding of spatiotemporal information within these neural networks. This can include exploring machine learning frameworks such as reinforcement learning (RL) and reservoir computing (RC), which may help unlock the next phases of OI. By scaffolding these experimental designs, we move to examine how organoids might evolve from simple neural assemblies to systems potentially capable of multimodal processing, intergenerational memory transfer, and polycomputational functionality—where multiple computational paradigms operate simultaneously within a unified system.

5.1 Layer 1: Organoid Array Computing

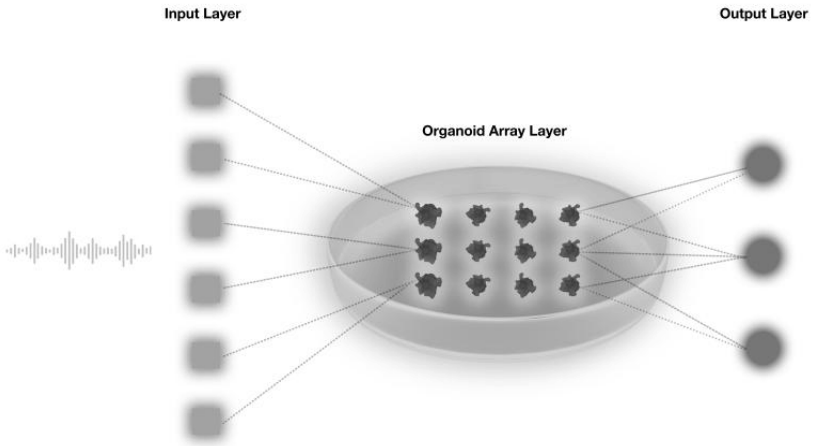

Figure 6 Design Layer 1: Organoid Array Computing. Input layer: the input layer converts information (image pattern, audio clips, time series, etc.) into various spatiotemporal sequences of electrical stimulation pulses; organoid array layer: the organoid array receives the input electrical stimulation and maps it to a high-dimensional computational space as the ONN; decoder layer: neural activities are fed into decoding functions such as linear regression or logistic regression to form an output layer for classification, recognition, and prediction. Figure by Jenn Leung.

Organoid array computing presents a biocomputing architecture that employs neural assemblies as physical computational systems. This approach aligns with the concept of RC, an energy-efficient method that utilizes an untrained reservoir and a linearly trained simple classifier.[77]

Our proposed design layer envisions a three-dimensional assembly of HBOs functioning as a nested ONN, serving as the reservoir in an RC framework for speech recognition tasks. Using a biological neural network as a form of reservoir in an RC framework, we could evaluate the feasibility of using a multi-organoid array as an RC system for speech recognition tasks.[78]

Building on recent advancements, including Brainoware's interface's success in vowel identification and Cortical Labs' exploration of multi-organoid arrays for memory storage, we devised potential future proof-of-concept for scaffolding for an expanding OI design space.[79] The set-up would include an array of HBOs, derived from induced pluripotent stem cells. This organoid array is housed in a custom-designed microfluidic platform that enables nutrient perfusion and potential interorganoid chemical signaling. Each organoid in the array is interfaced with a high-density microelectrode array (HD-MEA) for stimulation and recording. The entire system is maintained in an environmental control system to ensure optimal conditions for organoid health and function. A signal processing unit converts audio inputs into electrical stimuli, while a machine learning interface implements a linear classifier for decoding organoid responses (Figure 6).

We can consider the input layer as a layer that converts information (image pattern, audio clips, time series data) into various spatiotemporal sequences of electrical stimulation pulses that can be sent to the organoid.[80] It is shown in the DishBrain experiment that inputting electrophysiological input through eight stimulation electrodes with rate coding along with place coding electrical pulses to communicate

[75] Zhang et al., "Translational Organoid Technology."
[76] Bakkum et al., "Activity-Dependent Plasticity."
[77] Glover et al., "Reservoir Computing."
[78] Cai et al., "Brain Organoid Reservoir."
[79] Cai et al., "Brain Organoid Reservoir."
[80] Khajehnejad et al., "Biological Neurons."

bounded two-dimensional data is comparable and outperforms pixel-based information input to RL algorithms (Figure 7).[81] We can attempt to extend this experiment to cater for other inputs.

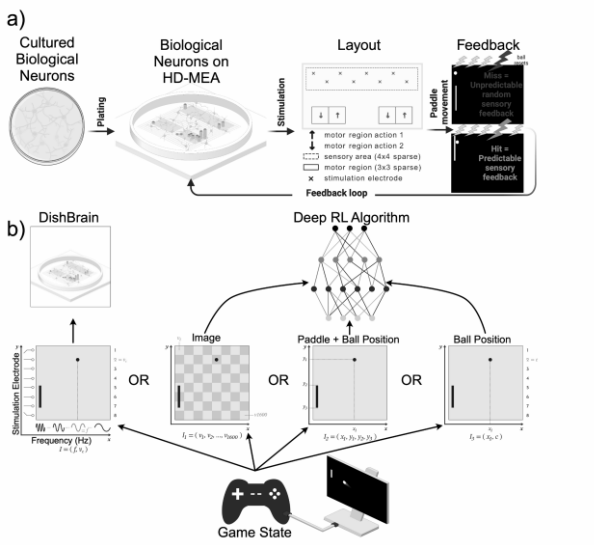

Figure 1: **DishBrain system and Various input designs to RL algorithms. a)** *DishBrain* feedback loop setup and Electrode configuration and predefined sensory and motor regions. Figures adapted and modified from (Kagan et al., 2022). **b)** Schematic comparing the information input routes in the *DishBrain* system (left) and the three implementations of the deep RL algorithms (right). In each design, the input information to the computing module (deep RL algorithms or *DishBrain*) is denoted by a vector I.

Figure 7 DishBrain system and various input designs to RL algorithms.[82]

In the organoid array computing layer, dozens of miniature HBOs are housed in the ONN, each serving as a node, each interfaced with an MEA and connected through a multichambered microfluidic device. As electrical stimuli (converted from audio inputs) are applied to the organoids via HD-MEAs, the complex neural networks within each organoid transform these inputs into a higher-dimensional representation (Figure 6). This is a fundamental property of RC, where the reservoir (in this case, the organoid array) projects the input into a high-dimensional space.

In the output/decoder layer, neural activities representing the state of the ONN are recorded by an MEA system and fed into decoding functions. This makes the output readable for downstream tasks, forming an output layer for classification, recognition, prediction, and other applications (Figure 6).[83]

The application of RC to OI expands the dimensions of biocomputing capabilities in the design space for experiments. As RC is a substrate-independent framework, it enables the expansive integration of various components into OI systems, including organoid arrays, microfluidic platforms, HD-MEAs, environmental control systems, signal processing units, and machine learning interfaces. The paper "Assembloid Learning" proposes that "personalized models" of neural assemblies could be developed in the near future for brain care and treatment optimization.[84] This possibility arises from the adaptive nature of neural assemblies, which can learn and respond to chemical and electrical stimuli that induce plastic changes, especially when derived from an individual's own cells.

The implementation of a multi-organoid RC framework in OI has several important implications for biocomputing. First, it enables assembloid learning, where multiple organoids can coordinate efforts, potentially mimicking the distributed processing of the human brain.[85] Second, the parallel processing capabilities of multiple organoids in an array could significantly increase computational capacity, enabling more complex task pooling and information processing, scaffolding for a task-pooling intelligence among organoids in the array. Additionally, as organoids have shown the ability to develop regional specialization similar to the human brain, this framework could lead to more sophisticated modeling of brain functions, leading to its expanding capabilities to process multimodal inputs.[86] Finally, the ability to process simulated environments, as demonstrated in experiments such as the "DishBrain" *Pong* game, opens up possibilities for creating virtual environments encoded as spatiotemporal electrophysiological activity.[87] These developments collectively suggest that OI has the potential to scaffold for multimodal and multilayered environments.

[81] Khajehnejad et al., "Biological Neurons."
[82] Khajehnejad et al., "Biological Neurons."
[83] Cai et al., "Brain Organoid Reservoir."
[84] Mencattini, "Assembloid Learning."
[85] Mencattini, "Assembloid Learning."
[86] Sun et al., "Translational Potential."
[87] Kagan et al., "In Vitro Neurons."

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by Jenn Leung, Chloe Loewith & Ivar Frisch
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5.2 Layer 2: Scaffolding for Multimodality

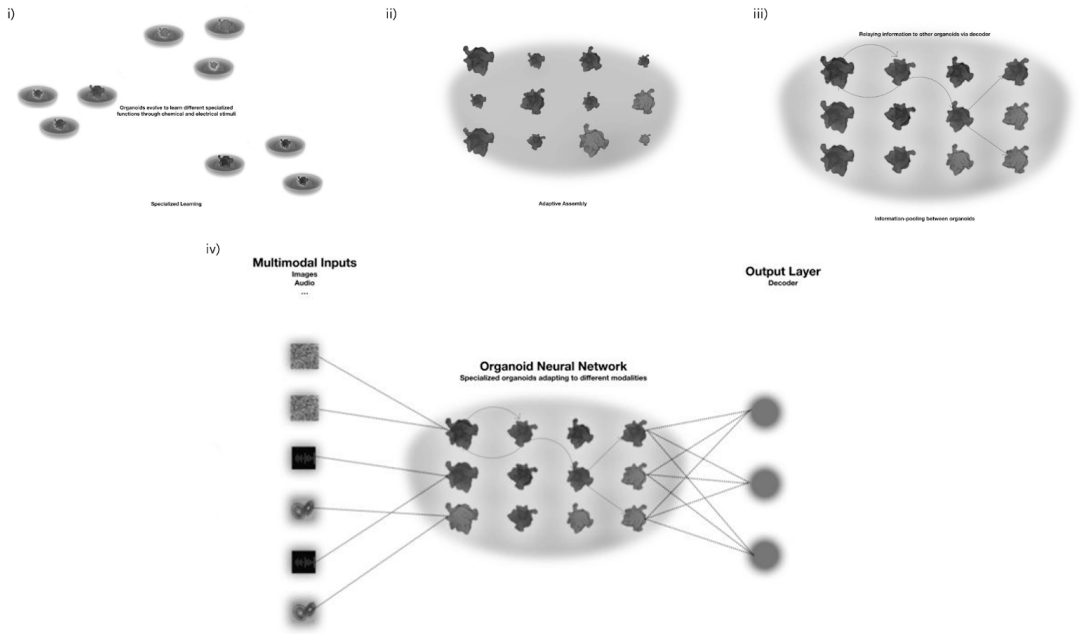

Figure 8 Design Layer 2: Multimodality Processing. (i) Organoids evolve to learn different specialized functions through chemical and electrical stimuli; (ii) adaptive assembly among HBOs; (iii) relaying information to other organoids via decoder; iv) Design Layer 2: Scaffolding for multimodality. Figure by Jenn Leung.

Building on the foundation of organoid array computing, we can envision a more advanced design layer that leverages the potential of multiple organoids to process multimodal inputs. Recent advancements in brain organoid research, such as region-specific HBOs, vascularized organoids, and assembloids (which combine different organoid types) provide a promising platform for this next step in OI.[88] By cultivating specialized structures that mimic the sensory regions of the brain, we can envision a system where different organoids within an array are designed to handle distinct types of sensory information (Figure 8).

For example, some HBOs could be guided to specialize in processing tailored inputs. This specialization could be achieved by exposing the organoids to specific inputs or stimulation techniques, such as electrical signals via MEAs, optogenetic manipulation, or chemical stimulation. With this approach, each organoid would act as a specialized processing unit, much like how the human brain allocates distinct regions to handle sensory inputs like sight, sound, and touch.[89]

If specialized organoids are developed, they could be integrated into an interconnected system where multimodal inputs are processed simultaneously. By employing different stimulation techniques to cater to each organoid's specialized function, the entire array would "collaborate" to interpret complex, multisensory inputs. This mirrors the brain's ability to integrate information from multiple sensory modalities to form a cohesive understanding of the environment.

Taking assembloid learning as a key path dependency from Layer 1, this layer suggests that HBOs could be cultivated to mimic the brain's inherent ability to process multimodal inputs. By utilizing one RC framework to handle this data, the organoid array would draw parallels between, for example, visual pixels, audio patterns, and touch-based stimuli. Such a system could extend the current capacities of OI, offering a design that not only processes individual data streams but also integrates them into a unified output. This paves the way for future experiments that explore how organoids could be trained to develop specialized functions adaptable to more complex, multimodal computing tasks.

[88] Schmidt, "Rise of the Assembloid" 2021; Mansour et al., "In Vivo Model" 2018; Susaimanickam et al., "Region Specific Brain Organoids." 2022
[89] Ackerman, Discovering the Brain.

5.3 Layer 3: Intergenerational Memory

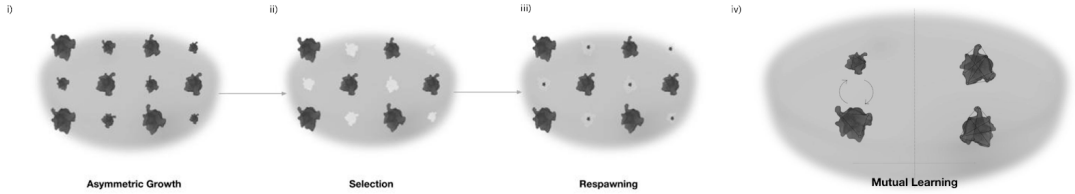

Figure 9 Design Layer 3: Intergenerational Memory. (i) Asymmetric growth with varying responses to different stimuli; (ii) HBOs go through a process of selection and survival throughout training process; (iii) respawning and optimizing HBOs; iv) mutual learning, where HBOs take into account the decoded response of other organoids during training, thereby growing new structures.

The speculative design space for intergenerational organoid communication is grounded in the emerging capabilities of HBOs to store and transmit complex information.[90] As organoids are increasingly capable of demonstrating aspects of memory storage and differentiation,[91] there is a potential for creating systems where these "memories" or learned behaviors could be passed from one organoid to another, or even across generations of organoids.

Intergenerational organoid communication could involve the transfer of encoded information from one organoid to another, simulating the biological inheritance of cognitive abilities. This could be achieved through advanced bioengineering platforms that enable the selective transfer of neural patterns, potentially akin to how synaptic plasticity underpins memory in the human brain (Figure 9).[92]

The potential applications of intergenerational memory in organoids could reconstruct how we understand and utilize biological intelligence. An organoid network could be designed where early-generation organoids undergo specific training or learning tasks, with subsequent generations inheriting this trained state, thus reducing the time and energy required for each new generation to achieve the same level of functionality. This would not only enhance the efficiency of organoid-based systems but could also lead to breakthroughs in understanding how memory and learning are encoded biologically. Such advancements might open new avenues for developing biocomputers that continuously evolve and adapt over time, integrating past experiences into future decision-making processes.

6 Implications

Advancements in organoid technologies have the potential to vastly benefit humanity. This research offers insights into human biology, disease mechanisms, and cognitive processes, and it holds promise for revolutionary medical treatments and drug development. Given these benefits, it would be misguided, and harmful, to prematurely halt or excessively restrict this field of study.

However, as we push the boundaries of what's possible with organoid development, we are presented with ethical challenges that require thoughtful ethical analysis. The potential emergence of morally relevant qualities, such as consciousness or advanced cognitive abilities, require due ethical consideration. Continued ethical assessment will be essential as this technology advances, requiring the establishment of clear criteria to identify morally relevant capacities in HBOs and guidelines for responding appropriately to their emergence. By doing so, we can advance this promising field responsibly, maximizing its benefits to humanity while mitigating ethical risks.

A recent letter titled "A Response to Claims of Emergent Intelligence and Sentience in a Dish" underscores the importance of cautious and precise language when describing the capabilities of neural systems.[93] The authors criticize the premature attribution of terms such as "sentience" and "intelligence" to neurons in a dish, emphasizing that such claims lack sufficient evidence and risk creating confusion around the ethical implications of this research. We must be vigilant in how we communicate OI's capabilities, ensuring that we do not oversell findings or trigger concerns before they are warranted

7 Conclusion

This paper considers the expansive design space of OI, providing a comprehensive application framework of OI. Our research has aimed to provide a taxonomy of design possibilities, considering various dimensions and parameters of inputs and outputs. These include alternative configurations of organoid assemblies, mechanical devices, microfluidics, bioprinting techniques, and culture media, all of which contribute to the complex ecosystem of OI. Although OI is still in its infancy, researchers could follow the design framework to devise experiments that are adaptable to the growing dimensions of

[90] Smirnova et al., "Organoid Intelligence: New Frontier."
[91] Cai et al., "Brain Organoid Reservoir" 2023; Smirnova et al., "Organoid Intelligence: New Frontier." 2023
[92] Kennedy, "Synaptic Signaling."
[93] Balci et al., "Response to Claims."

DOI 10.1162/ANTI.5CZM

neural assemblies, facilitating plastic changes during the development of research. This plasticity enables the creation of personalized models for OI, which could revolutionize our approach to understanding and manipulating these intricate biological systems.

As researchers face the challenge of isolating and monitoring specific inputs, outputs, and developmental stages to gain a deeper understanding of how HBOs function, the design space points to the nested complex system as a growing and self-patterning hybrid entity.

While the field of OI often emphasizes the evolved rather than designed nature of intelligence, it's important to note the growing body of research in evolutionary computation and artificial life. These fields reveal the potential for mechanical and refractive computing in various substrates, including granular matter.

Ultimately, the future of OI lies in the integration of multiple computational paradigms. Chemical computing, biological computing, and scaffolding systems together create a nested, multiscale system for OI. With the integration of diverse computational approaches such as RC with living neural networks, we are starting to understand forms of intelligence that more closely mirror the complexity of biological brains. To imagine the future of OI, we must also imagine growing cognitive assemblies with the capacity for physical evolution. Unlike traditional computing systems with fixed hardware architectures, organoid arrays can grow, adapt, and reorganize their physical structure in response to computational demands.

Bibliography

Ackerman, Sandra, and National Academy Institute of Medicine. *Discovering the Brain*. National Academies Press, 1992.

Bagley, Joshua A., Daniel Reumann, Shan Bian, Julie Lévi-Strauss, and Juergen A. Knoblich. "Fused Cerebral Organoids Model Interactions between Brain Regions." *Nature Methods* 14, no. 7 (July 2017): 743–51. https://doi.org/10.1038/nmeth.4304.

Bakkum, Douglas J., Zenas C. Chao, and Steve M. Potter. "Long-Term Activity-Dependent Plasticity of Action Potential Propagation Delay and Amplitude in Cortical Networks." *PLoS One* 3, no. 5 (May 2008): e2088. https://doi.org/10.1371/journal.pone.0002088.

Balci, Fuat, Suliann Ben Hamed, Thomas Boraud, et al. "A Response to Claims of Emergent Intelligence and Sentience in a Dish." *Neuron* 111, no. 5 (March 2023): 604–5. https://doi.org/10.1016/j.neuron.2023.02.009.

Baldassari, Simona, Ilaria Musante, Michele Iacomino, Federico Zara, Vincenzo Salpietro, and Paolo Scudieri. "Brain Organoids as Model Systems for Genetic Neurodevelopmental Disorders." *Frontiers in Cell and Developmental Biology* 8 (October 2020): 590119. https://doi.org/10.3389/fcell.2020.590119.

Ballav, Sangeeta, Amit Ranjan, Shubhayan Sur, and Soumya Basu. "Organoid Intelligence: Bridging Artificial Intelligence for Biological Computing and Neurological Insights." In *Technologies in Cell Culture – A Journey From Basics to Advanced Applications*, edited by Soumya Basu, Amit Ranjan, and Subhayan Sur. IntechOpen, June 2024. https://doi.org/10.5772/intechopen.114304.

Beaulieu, Shawn, Piper Welch, Atoosa Parsa, Corey O'Hern, Rebecca Kramer-Bottligio, and Josh Bongard. "Refractive Computation: Parallelizing Logic Gates across Driving Frequencies in a Mechanical Polycomputer." In *ALIFE '24: Proceedings of the 2024 Artificial Life Conference*, edited by Andrés Faíña, Sebastian Risi, Eric Medvet, et al. MIT Press, 2024. https://doi.org/10.1162/isal_a_00807.

Birey, Fikri, Jimena Andersen, Christopher D. Makinson, et al. "Assembly of Functionally Integrated Human Forebrain Spheroids." *Nature* 545, no. 7652 (May 2017): 54–59. https://doi.org/10.1038/nature22330.

Blakemore, Erin. "Human-Pig Hybrid Created in the Lab—Here Are the Facts." *National Geographic*, January 26, 2017. https://www.nationalgeographic.com/science/article/human-pig-hybrid-embryo-chimera-organs-health-science.

Bongard, Joshua, and Michael Levin. "Living Things Are Not (20th Century) Machines: Updating Mechanism Metaphors in Light of the Modern Science of Machine Behavior." *Frontiers in Ecology and Evolution* 9 (March 2021): 650726. https://doi.org/10.3389/fevo.2021.650726.

———. "There's Plenty of Room Right Here: Biological Systems as Evolved, Overloaded, Multi-Scale Machines." *Biomimetics* 8, no. 1 (March 2023): 110. https://doi.org/10.3390/biomimetics8010110.

Cai, Hongwei, Zheng Ao, Chunhui Tian, et al. "Brain Organoid Reservoir Computing for Artificial Intelligence." *Nature Electronics* 6, no. 12 (December 2023): 1032–39. https://doi.org/10.1038/s41928-023-01069-w.

Chen, H. Isaac, John A. Wolf, Rachel Blue, Mingyan Maggie Song, Jonathan D. Moreno, Guo-Li Ming, and Hongjun Song. "Transplantation of Human Brain Organoids: Revisiting the Science and Ethics of Brain Chimeras." *Cell Stem Cell* 25, no. 4 (October 2019): 462–72. https://doi.org/10.1016/j.stem.2019.09.002.

Chung, Won Gi, Enji Kim, Hayoung Song, et al. "Recent Advances in Electrophysiological Recording Platforms for Brain and Heart Organoids." *Advanced NanoBioMed Research* 2, no. 1 (December 2022): 2200081. https://doi.org/10.1002/anbr.202200081.

Cogoni, Florian, David Bernard, Roxana Kazhen, Salvatore Valitutti, Valérie Lobjois, and Sylvain Cussat-Blanc. “ISiCell: A Participatory Methodology and Platform for Collaborative Agent-Based Modeling in Cell Biology.” In *ALIFE ’24: Proceedings of the 2024 Artificial Life Conference*, edited by Andrés Faíña, Sebastian Risi, Eric Medvet, et al. MIT Press, 2024.

Cronin, Leroy. “The Chemputer and Chemputation: A Universal Chemical Compound Synthesis Machine.” Preprint, arXiv, August 17 2024. https://doi.org/10.48550/arXiv.2408.09171.

Emulate. “Brain-Chip.” Accessed May 2021. https://emulatebio.com/brain-chip/.

Fernandes, Tiago G. “Organoids as Complex (Bio)Systems.” *Frontiers in Cell and Developmental Biology* 11 (August 2023): 1268540. https://doi.org/10.3389/fcell.2023.1268540.

Final Spark. “Neuroplatform – FinalSpark.” Accessed April 2024. https://finalspark.com/neuroplatform/.

Friston, Karl. “The Free-Energy Principle: A Unified Brain Theory?” *Nature Reviews Neuroscience* 11, no. 2 (February 2010): 127–38. https://doi.org/10.1038/nrn2787.

Gabriel, Elke, Walid Albanna, Giovanni Pasquini, et al. “Human Brain Organoids Assemble Functionally Integrated Bilateral Optic Vesicles.” *Cell Stem Cell* 28, no. 10 (October 2021): 1740–1757.e8. https://doi.org/10.1016/j.stem.2021.07.010.

Glover, Tom, Evgeny Osipov, and Stefano Nichele. “On When Is Reservoir Computing with Cellular Automata Beneficial?” Preprint, arXiv, June 13, 2024. https://doi.org/10.48550/arXiv.2407.09501.

Goldman, Bruce. “Here Come the Assembloids.” *Stanford Medicine Magazine*, July 27, 2022. https://stanmed.stanford.edu/brain-tissue-assembloids-expand-brain-understanding/.

Greenberg, Alissa. “The Birth of Wetware.” *proto.life*, May 2018. https://proto.life/2018/05/the-birth-of-wetware/.

Hyun, Insoo, J. C. Scharf-Deering, and Jeantine E. Lunshof. “Ethical Issues Related to Brain Organoid Research.” *Brain Research* 1732 (April 2020): 146653. https://doi.org/10.1016/j.brainres.2020.146653.

Jaiswal, Ashish, Ashwin Ramesh Babu, Mohammad Zaki Zadeh, Debapriya Banerjee, and Fillia Makedon. “A Survey on Contrastive Self-Supervised Learning.” *Technologies* 9, no. 1 (2021): 2. https://doi.org/10.3390/technologies9010002.

Kagan, Brett J., Andy C. Kitchen, Nhi T. Tran, et al. “In Vitro Neurons Learn and Exhibit Sentience When Embodied in a Simulated Game-World.” *Neuron* 110, no. 23 (December 2022): 3952–3969.e8. https://doi.org/10.1016/j.neuron.2022.09.001.

Kengla, Carlos, Anthony Atala, and Sang Jin Lee. “Bioprinting of Organoids.” In *Essentials of 3D Biofabrication and Translation*, edited by Anthony Atala and James J. Yoo. Elsevier, 2015.

Kennedy, Mary B. “Synaptic Signaling in Learning and Memory.” *Cold Spring Harbor Perspectives in Biology* 8, no. 2 (December 2013): a016824. https://doi.org/10.1101/cshperspect.a016824.

Khajehnejad, Moein, Forough Habibollahi, Aswin Paul, Adeel Razi, and Brett J. Kagan. “Biological Neurons Compete with Deep Reinforcement Learning in Sample Efficiency in a Simulated Gameworld.” Preprint, arXiv, May 27, 2024. https://doi.org/10.48550/arXiv.2405.16946.

Kirihara, Takaaki, Zhongyue Luo, Siu Yu A. Chow, et al. “A Human Induced Pluripotent Stem Cell-Derived Tissue Model of a Cerebral Tract Connecting Two Cortical Regions.” *iScience* 14 (April 2019): 301–11. https://doi.org/10.1016/j.isci.2019.03.012.

Kruszelnicki, Karl S. “Mouse with Human Ear.” *ABC Science*, June 2, 2006. https://www.abc.net.au/science/articles/2006/06/02/1644154.htm.

Levy, Rebecca J., and Sergiu P. Paşca. “What Have Organoids and Assembloids Taught Us About the Pathophysiology of Neuropsychiatric Disorders?” *Biological Psychiatry* 93, no. 7 (April 2023): 632–41.

Makrygianni, Evanthia A., and George P. Chrousos. “From Brain Organoids to Networking Assembloids: Implications for Neuroendocrinology and Stress Medicine.” *Frontiers in Physiology* 12 (June 2021): 621970. https://doi.org/10.3389/fphys.2021.621970.

Mansour, Abed Alfatah, J. Tiago Gonçalves, Cooper W. Bloyd, et al. “An In Vivo Model of Functional and Vascularized Human Brain Organoids.” *Nature Biotechnology* 36, no. 5 (June 2018): 432–41. https://doi.org/10.1038/nbt.4127.

Mencattini, Arianna, Elena Daprati, David Della-Morte, Fiorella Guadagni, Federica Sangiuolo, and Eugenio Martinelli. “Assembloid Learning: Opportunities and Challenges for Personalized Approaches to Brain Functioning in Health and Disease.” *Frontiers in Artificial Intelligence* 7 (April 2024): 1385871. https://doi.org/10.3389/frai.2024.1385871.

Muotri, Alysson R. “Brain Model Technology and Its Implications.” *Cambridge Quarterly of Healthcare Ethics 32, no. 4* (2023): 597–601. https://doi.org/10.1017/S096318012300018X.

Osaki, Tatsuya, Tomoya Duenki, Siu Yu A. Chow, et al. “Complex Activity and Short-Term Plasticity of Human Cerebral Organoids Reciprocally Connected with Axons.” *Nature Communications* 15, no. 1 (April 2024): 2945. https://doi.org/10.1038/s41467-024-46787-7.

Parisi, German I., Ronald Kemker, Jose L. Part, Christopher Kanan, and Stefan Wermter. “Continual Lifelong Learning with Neural Networks: A Review.” *Neural Networks* 113 (2019): 54–71. https://doi.org/10.1016/j.neunet.2019.01.012.

Passaro, Austin P., and Steven L. Stice. “Electrophysiological Analysis of Brain Organoids: Current Approaches and Advancements.” *Frontiers in Neuroscience* 14 (2020): 622137. https://doi.org/10.3389/fnins.2020.622137.

Quadrato, Giorgia, Tuan Nguyen, Evan Z. Macosko, et al. “Cell Diversity and Network Dynamics in Photosensitive Human Brain Organoids.” *Nature* 545, no. 7652 (May 2017): 48–53. https://doi.org/10.1038/nature22047.

Quintard, Clément, Emily Tubbs, Gustav Jonsson, et al. “A Microfluidic Platform Integrating Functional Vascularized Organoids-on-Chip.” *Nature Communications* 15, no. 1 (February 2024): 1452. https://doi.org/10.1038/s41467-024-45710-4.

Ren, Ya, Xue Yang, Zhengjiang Ma, et al. “Developments and Opportunities for 3D Bioprinted Organoids.” *International Journal of Bioprinting* 7, no. 3 (June 2021): 364. http://doi.org/10.18063/ijb.v7i3.364.

Reumann, Daniel, Christian Krauditsch, Maria Novatchkova, et al. “In Vitro Modeling of the Human Dopaminergic System Using Spatially Arranged Ventral Midbrain-Striatum-Cortex Assembloids.” *Nature Methods* 20, no. 12 (December 2023): 2034–47. https://doi.org/10.1038/s41592-023-02080-x.

Richards, Blake A., and Timothy P. Lillicrap. “The Brain-Computer Metaphor Debate Is Useless: A Matter of Semantics.” *Frontiers in Computational Science* 4 (February 2022): 810358. https://doi.org/10.3389/fcomp.2022.810358.

Sato, Toshiro, Robert G. Vries, Hugo J. Snippert, et al. “Single Lgr5 Stem Cells Build Crypt-Villus Structures in Vitro without a Mesenchymal Niche.” *Nature* 459, no. 7244 (May 2009): 262–65. https://doi.org/10.1038/nature07935.

Schmidt, Charlie. “The Rise of the Assembloid.” *Nature* 597, no. 7878 (September 2021): S22–23. https://doi.org/10.1038/d41586-021-02628-x.

Sha, Xin Wei. “Metabolic Approach to Designing Space.” In *The Space of Technicity, Theorising Social, Technical and Environmental Entanglements*, edited by Robert Gorny, Stavros Kousoulas, Dulmini Perera, and Andrej Radman. TU Delft OPEN Publishing and Jap Sam Books, 2024.

Sharf, Tal, Tjitse van der Molen, Stella M. K. Glasauer, et al. “Functional Neuronal Circuitry and Oscillatory Dynamics in Human Brain Organoids.” *Nature Communications* 13, no. 1 (July 2022): 4403. https://doi.org/10.1038/s41467-022-32115-4.

Shi, Yingchao, Le Sun, Mengdi Wang, et al. “Vascularized Human Cortical Organoids (vOrganoids) Model Cortical Development in Vivo.” *PLoS Biology* 18, no. 5 (May 2020): e3000705. https://doi.org/10.1371/journal.pbio.3000705.

Skylar-Scott, Mark A., Jeremy Y. Huang, Aric Lu, et al. “An Orthogonal Differentiation Platform for Genomically Programming Stem Cells, Organoids, and Bioprinted Tissues.” Preprint, bioRxiv, July 2020. http://dx.doi.org/10.1101/2020.07.11.198671.

Smirnova, Lena, Brian S. Caffo, David H. Gracias, et al. “Organoid Intelligence (OI): The New Frontier in Biocomputing and Intelligence-in-a-Dish.” *Frontiers in Science* 1 (February 2023): 1017235. https://doi.org/10.3389/fsci.2023.1017235.

Smirnova, Lena, Itzy E. Morales Pantoja, and Thomas Hartung. “Organoid Intelligence (OI) - The Ultimate Functionality of a Brain Microphysiological System.” *ALTEX* 40, no. 2 (2023): 191–203. https://doi.org/10.14573/altex.2303261.

Sun, Alfred X., Huck-Hui Ng, and Eng-King Tan. “Translational Potential of Human Brain Organoids.” *Annals of Clinical and Translational Neurology* 5, no. 2 (February 2018): 226–35. https://doi.org/10.1002/acn3.505.

Sun, Nan, Xiangqi Meng, Yuxiang Liu, Dan Song, Chuanlu Jiang, and Jinquan Cai. “Applications of Brain Organoids in Neurodevelopment and Neurological Diseases.” *Journal of Biomedical Science* 28, no. 1 (April 2021): 30. https://doi.org/10.1186/s12929-021-00728-4.

Suong, Dang Ngoc Anh, Keiko Imamura, Yoshikazu Kato, and Haruhisa Inoue. “Design of Neural Organoids Engineered by Mechanical Forces.” *IBRO Neuroscience Reports* 16 (June 2024): 190–95. https://doi.org/10.1016/j.ibneur.2024.01.004.

Susaimanickam, Praveen Joseph, Ferdi Ridvan Kiral, and In-Hyun Park. “Region Specific Brain Organoids to Study Neurodevelopmental Disorders.” *International Journal of Stem Cells* 15, no. 1 (February 2022): 26–40. https://doi.org/10.15283/ijsc22006.

Takahashi, Kazutoshi, and Shinya Yamanaka. “Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors.” *Cell* 126, no. 4 (August 2006): 663–76. https://doi.org/10.1016/j.cell.2006.07.024.

The Tissue Culture & Art Project. “Crude Matter.” 2012. https://tcaproject.net/portfolio/crude-matter/.

Trujillo, Cleber A., Edward S. Rice, Nathan K. Schaefer, et al. “Reintroduction of the Archaic Variant of *NOVA1* in Cortical Organoids Alters Neurodevelopment.” *Science* 371, no. 6530 (February 2021): eaax2537. https://doi.org/10.1126/science.aax2537.

Tsanni, Abdullahi. “Human Brain Cells Hooked Up to a Chip Can Do Speech Recognition.” *MIT Technology Review*, December 11, 2023. https://www.technologyreview.com/2023/12/11/1084926/human-brain-cells-chip-organoid-speech-recognition/.

Wang, Yanfang, Jiejie Wang, Ziyu Ji, et al. “Application of Bioprinting in Ophthalmology.” *International Journal of Bioprinting* 8, no. 2 (February 2022): 552. https://doi.org/10.18063/ijb.v8i2.552.

Zabolocki, Michael, Kasandra McCormack, Mark van den Hurk, et al. “BrainPhys Neuronal Medium Optimized for Imaging and Optogenetics in Vitro.” *Nature Communications* 11, no. 1 (November 2020): 5550. https://doi.org/10.18063/ijb.v8i2.552.

Zhang, Shun, Zhengpeng Wan, and Roger D. Kamm. “Vascularized Organoids on a Chip: Strategies for Engineering Organoids with Functional Vasculature.” *Lab on a Chip* 21, no. 3 (February 2021): 473–88. https://doi.org/10.1039/d0lc01186j.

Zhang, Weijie, Jiawei Li, Jiaqi Zhou, Abhay Rastogi, and Shaohua Ma. “Translational Organoid Technology – The Convergence of Chemical, Mechanical, and Computational Biology.” *Trends in Biotechnology* 40, no. 9 (September 2022): 1121–35. https://doi.org/10.1016/j.tibtech.2022.03.003.