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REVIEW ARTICLE

When Is a Brain Organoid Ready to Learn and Think?

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Abstract

Brain organoids, initially developed as models of human neurodevelopment, are increasingly being integrated with recording, stimulation, and computational systems to explore biological computing. However, most studies assess computational performance only after organoids have been selected and trained. A key unresolved question is whether pre-training electrophysiological features can predict an individual organoid's capacity for computational learning. Here, we define computational learnability as reproducible and partially generalizable task improvement under a specified input, feedback, interface, and decoding protocol, with a demonstrable contribution from the biological system. We critically review the limited evidence for pre-training prediction and examine candidate electrophysiological predictors spanning signal quality, spontaneous network dynamics, evoked responses, plasticity, and stability. Their predictive value is likely to depend on the computational task and interface rather than converge on a universal measure. We further outline key requirements for validation, including prospective prediction in previously unseen organoids, rigorous separation of biological learning from maturation, repeated stimulation, interface drift, and decoder adaptation, and appropriate control of batch effects and uncertainty. Establishing robust predictors across organoids, batches, tasks, interfaces, and laboratories will be essential for developing electrophysiology-based functional screening strategies for brain organoid computing.

Keywords

brain organoid; organoid intelligence; electrophysiology; computational learnability; multielectrode array; functional quality control; predictive biomarker; closed-loop learning

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1. Introduction

Brain organoids were developed as three-dimensional models of human brain development and neurological disease [1-4]. Research has since moved beyond structure alone and now measures neural function directly. Human brain organoids can contain neural and glial cells, respond to stimulation, generate spontaneous network activity, produce complex oscillations, and form working neural circuits [5-8]. These properties do not make an organoid equivalent to a human brain. They show, more narrowly, that its neural state can be recorded and changed, which allows the tissue to be studied as a possible biological system for information processing.

This functional work supports the emerging field of organoid intelligence (OI), in which neural organoids are connected to systems for recording, stimulation, and computation [9-11]. Brainoware, for example, used nonlinear responses and fading memory as a biological reservoir [12]. Automated systems such as Neuroplatform made longer recordings and closed-loop experiments feasible [13-14]. In the cartpole study, mouse cortical organoids improved under the tested training conditions, but the effect was absent after a 45-minute rest [15]. The same study found that causal connectivity measured before the task was linked to later performance. Other human neural organoid systems have shown functional connectivity, dynamics related to criticality, and plasticity caused by stimulation [16]. A preprint using paired organoids also reported network-level long-term potentiation and improvement under feedback in a maze-like task [17], but this work has not been peer reviewed. Together, these studies identify neural dynamics that may be useful for computation and may contain predictive information before training. They do not establish independent, standardized, or general learning across tasks.

Existing reviews discuss OI, organoid computing, electrophysiological methods, and neural interfaces [18-20]. This review focuses on the unresolved step between describing neural activity and using it to make a decision before training: can baseline electrophysiology guide sample selection and functional quality control? The problem is difficult because the observed outcome belongs to a biohybrid system rather than to the organoid alone. Recordings from a single organoid contain measurements from many electrodes and time windows, while several organoids can belong to one differentiation batch. The digital decoder may also change during the experiment. An observed gain can therefore combine plasticity or maturation with interface drift, repeated stimulation, and decoder optimization.

Biological variation adds a separate source of uncertainty. Interface design partly determines which organoid activity enters the recording [20]. The substrate itself varies across donors and cell lines and with differentiation batch, organoid age, and cell composition [21-25]; culture conditions and production methods introduce further variation [26-30]. Existing quality-control procedures assess identity, shape, composition, and production consistency. Yet an organoid that meets those criteria may still give a weak or unstable signal, or one that cannot be clearly distinguished. Functional quality control must establish both whether the organoid was produced correctly and whether its dynamics are reliable and informative for the intended task.

In this review, computational learnability is the ability of a brain organoid to show reproducible

improvement in computational performance under a defined input, feedback, interface, and decoding protocol. At least part of the improvement must come from the biological system, and some of it must extend beyond the training data. This definition is intentionally narrower than activity, maturity, responsiveness, plasticity, or strong performance at one time point. It is also task dependent. For example, an organoid that works well for temporal prediction may not be the best choice for closed-loop control.

The available direct evidence is narrow. A 2026 study of mouse cortical organoids found that connectivity measured before the task was associated with later performance [15], but the result comes from one platform and one task. No multifeature predictor has yet been fixed before analysis and tested externally across independent human organoids, batches, tasks, interfaces, and laboratories. We therefore treat readiness as a practical prediction problem rather than a search for one decisive biomarker. Biological condition, interface quality, spontaneous activity, probe response, and the task outcome all need to be considered before a prediction can support a decision.

Figure 1 brings these elements together. The review begins by defining the biohybrid system and the evidence needed to establish computational learnability. It then follows a simple path from the organoid and interface to network activity, probe response, task prediction, and validation. The final sections explain how controls separate biological learning from apparent improvement and how a predictor could move from proof of concept to functional quality control.

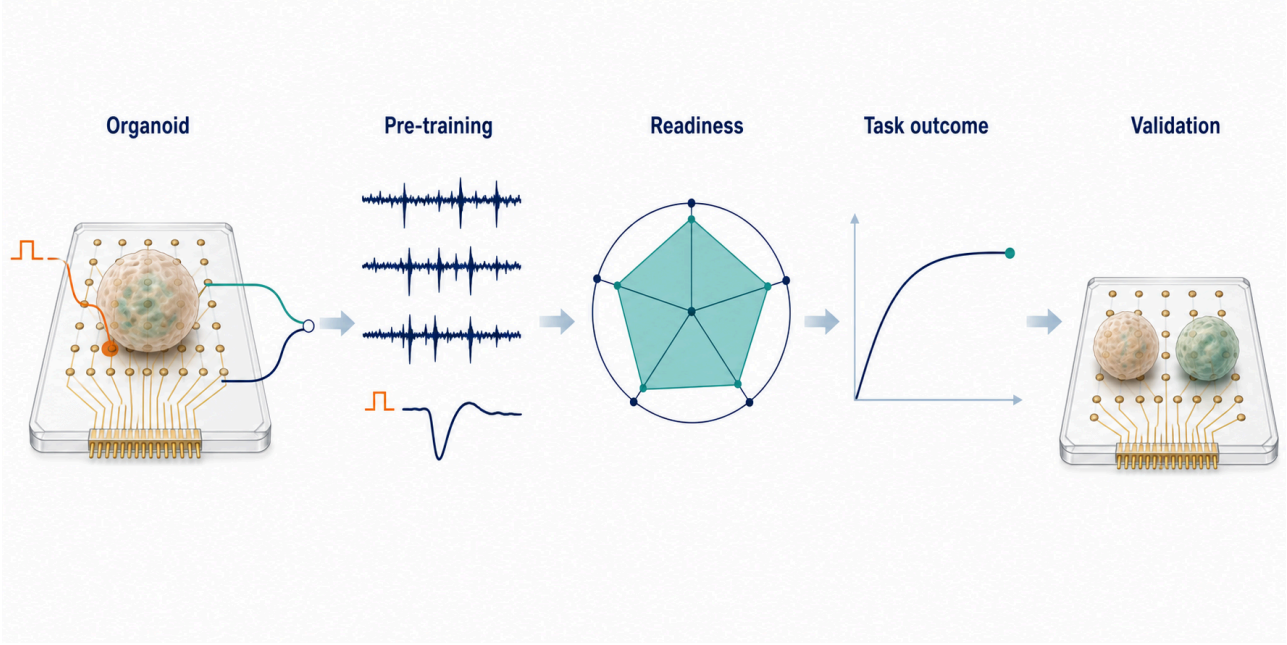


Figure 1. Conceptual overview of pre-training prediction in a biohybrid brain-organoid system.

2. Defining the prediction problem in a biohybrid system

2.1 *From tissue identity to network function*

A biological system needs more than living neurons to be useful for computing. Its networks must respond to input, produce state changes that are rich enough for computation, and remain stable over the period being studied. As they develop, brain organoids generate neuronal and glial cell types, form synaptic structures, and begin to show spontaneous electrical activity and network-level oscillations [4-8]. Microglia-like cells can further support neuronal function in some cerebral organoid systems [31]. These electrical patterns keep changing during maturation and do not settle into a fixed organization [6,8,32,33]. Noninvasive imaging also shows continued neuronal and oligodendroglial development over time [34]. Researchers have reduced variation by improving organoid uniformity and structural control [26-30]. Microenvironmental support, fluid handling, access to smaller networks, and standardized organoid models provide related strategies [35-38]. These changes may improve experimental control, but they also alter the tissue that is eventually measured. Culture age and production method are therefore part of the computational setting, not just background information. They shape the signal distribution, effective connectivity, and time integration available to a task.

Development over time can make task results difficult to interpret. When training lasts for hours or days, better performance may occur alongside maturation, a changing excitation-inhibition balance, altered cell viability, or movement at the electrode interface. Researchers need to establish both that the organoid has functional neural activity and that this activity remains stable enough for a meaningful comparison. Complex dynamics may be biologically interesting, but they are of limited computational use if they cannot be measured again.

Plasticity is another requirement, although it is not the same as learning. Thalamocortical assembloids and neural organoid microphysiological systems have shown short- and long-term changes after stimulation that are consistent with synaptic plasticity [16,39]. These findings provide a possible biological basis for training. On their own, however, they do not show goal-directed learning; the measured outcome may be a change in an evoked response rather than better performance on a separate test task. We therefore treat plasticity as a candidate mechanism and predictor, not as a substitute for evidence of learning.

2.2 *What related biological and digital systems establish*

OI has borrowed several experimental ideas from two-dimensional neuronal cultures. Following consensus nomenclature, we treat neuronal cultures, spheroids, organoids, and assembloids as related but non-equivalent systems [40]. DishBrain connected human and mouse neural cultures to a simulated Pong environment and reported task-related change under closed-loop feedback [41]. Earlier work had used closed-loop MEA stimulation to control bursting in cortical cultures [42]. Later studies linked structured information input to critical dynamics [43], measured information-processing capacity [44], or applied online supervised learning to temporal patterns [45]. Together with broader work on human in vitro synaptic function, these studies show how embodied environments, changing stimulation, and numerical task outcomes can be investigated [46]. They are

useful supporting evidence, but they are not direct evidence from organoids.

The biological substrate still matters. DishBrain did not use brain organoids, and a three-dimensional organoid differs from a monolayer in cellular organization, diffusion, maturation, access to electrodes, and recording depth. Results from monolayers can motivate controls or candidate features, but they cannot establish the same effect in a human brain organoid. Organoids are not automatically better reservoirs either. Their complexity may provide useful dynamics, yet it also introduces variability, hidden states, interface instability, and additional experimental cost.

Brainoware illustrates the combined biological and digital design of current organoid computing [12]. Encoded electrical inputs were transformed by the organoid into high-dimensional responses over time, and a digital readout interpreted those responses. The resulting system is neither fully biological nor fully digital. Its performance depends on input encoding, organoid dynamics, electrode sampling, feature construction, readout training, and evaluation design. To estimate what the organoid adds, studies need comparisons with shuffled signals, random or untrained reservoirs, digital-only baselines, and standard reservoir computers.

2.3 *Interfaces shape the observable signal*

Several interface designs now provide access to a larger portion of the organoid. Three-dimensional shell, multifunctional, CMOS, and microneedle systems extend surface access and recording density [47-51]. Mesh and embedded arrays support chronic or depth-resolved recording [52,53]. High-density CMOS and multilayer arrays extend surface mapping or depth access [54,55]. Long-term high-density MEA studies also show that matching the same cell or recording site across time is an analytical problem, not an automatic benefit of recording for longer [56]. Some bioelectronic systems can alter ions or neurotransmitters while recording activity [57]. Because of this, the interface cannot be treated as a neutral observer. Increasing array density may make the recorded state seem more dimensional, while broader surface coverage can make synchrony easier to detect. Evoked responses also depend on stimulation geometry, and long-term stability depends partly on how the tissue contacts the electrodes. Any computational phenotype must be interpreted in relation to the platform that produced it.

This dependence on the platform has two practical consequences. First, a raw feature threshold may need recalibration before it is transferred to another system. Second, measurement details belong in the model as predictors or grouping variables rather than being left as administrative notes. Studies of readiness biomarkers should report the electrode type, sampling rate, preprocessing, active-channel criteria, stimulus amplitude, organoid position, and recording duration.

Recent interface studies show the range of measurements that is now technically possible. In 2026, a shape-conformal porous framework provided nearly complete surface coverage through 240 independently addressable electrodes and supported spatial recording, programmed stimulation, imaging, and optogenetic control [58]. A stretchable microneedle microelectrode array produced a higher signal-to-noise ratio and more stable long-term recordings than planar configurations in human cerebral organoids [59]. Multilayer arrays have also recorded activity at different depths for more than four weeks [55]. These results make dense, longitudinal, three-dimensional measurement

feasible. They do not yet make features comparable across platforms or establish standardized thresholds and predictive validity.

2.4 *The organoid, interface, and decoder form one measurement pipeline*

OI platforms convert biological activity into repeated observations at several linked levels. Neuroplatform shows how quickly data volume grows when recordings are continuous and experiments can be run remotely [13]. A researcher cannot inspect all of these data by hand. Storage is less problematic than representation: the analysis must preserve biological variation, avoid treating technical noise as biology, and keep model-development information out of the evaluation.

An OI experiment is a chain of dependent operations. Biological preparation, sensing or stimulation, preprocessing, feature construction or representation learning, digital decoding, feedback, and validation all transform the information in some way. Each can also fail differently. Weak activity may come from an unhealthy organoid, whereas apparent drift may come from unstable tissue-electrode contact. Preprocessing can remove a real temporal pattern, and a flexible decoder can learn noise that does not recur in new data. A nominally closed loop may even deliver feedback that is unrelated to behavior. At the validation stage, treating windows from one organoid as independent samples creates a further error. Data science cannot correct poor biology or engineering, but it can help locate the source of measured performance.

2.5 *From neural activity to computational learnability*

Several terms used in OI describe different levels of evidence. Neural activity means that electrical events are present, and responsiveness means that stimulation produces a consistent change. Adaptation is a change in response over time, whereas plasticity is a lasting change in neural function linked to earlier activity. Task learning requires improvement toward a defined goal and a suitable counterfactual, meaning a comparison that represents what would have happened under another condition. Retention asks whether the improvement remains after training stops; generalization asks whether it transfers to new inputs or conditions. Using these terms as synonyms makes the evidence appear stronger than it is.

At the lowest level, a response to stimulation shows only that the input reached the network. A change across repeated trials can still arise from habituation, sensitization, fatigue, or short-term plasticity. The evidence becomes stronger when improvement depends on meaningful feedback because the change is then linked to the relation between an output and its consequence. Retention and performance on new inputs strengthen the claim further. For the strongest current claim, the effect should also be reproduced in independent organoids or batches, and the digital decoder alone should not produce the same improvement.

Table 1 turns this evidence ladder into operational distinctions. For each level, it states which broader claim the available evidence still cannot support.

Table 1. Evidence ladder for operational claims in organoid intelligence

Evidence level	Minimum operational evidence	Conclusion that remains unsupported
1 Neural activity	Detectable spontaneous or evoked electrical events above an artifact-controlled threshold	Learning, memory, intelligence, or consciousness
2 Responsiveness	Reproducible change after a defined input compared with baseline or sham input	Persistent adaptation or task learning
3 Plasticity	Activity-dependent change that persists beyond the immediate response and is compared with an appropriate control	Goal-directed improvement or generalization
4 Task improvement	Performance changes relative to a defined objective and baseline	Biological learning if decoder optimization and drift are uncontrolled
5 Feedback-dependent adaptation	Improvement exceeds random, noncontingent, or replay feedback under matched exposure	Long-term retention or task-general intelligence
6 Computational learnability	Reproducible, biologically attributable, and partly generalizable improvement under a specified protocol	Universal or human-equivalent intelligence

2.6 *What current studies establish and what controls are still needed*

Published studies reach different points on this evidence ladder. Brainoware showed that organoid dynamics can be useful for computation, but its reservoir design did not demonstrate independent learning by the organoid [12]. DishBrain and later studies using supervised feedback provide related evidence for task adaptation in two-dimensional cultures [41,45]. Robbins and colleagues reported that mouse cortical organoids improved under feedback relative to random or absent training signals [15]. The effect disappeared after rest and depended on glutamatergic transmission; connectivity measured before the task was also associated with later performance. A recent preprint using paired human organoids reported network-level long-term potentiation, drug-related changes, and improvement under feedback in a maze-like task [17]. Because that work has not been peer reviewed, the Robbins study remains the strongest direct peer-reviewed evidence for prediction before training. Its scope is nevertheless limited to one species, one task, one platform, and one analysis pipeline, and it did not test a prespecified biomarker in an independent dataset.

Calling this "learning" is only meaningful when the term is defined operationally. The definition needs to identify the biological system and task, describe how behavior determines feedback, and state the comparison group, outcome, training duration, and retention period. If these details are missing, several different effects may be grouped under "learning," making the claim stronger than the evidence allows.

Table 2 summarizes how representative systems differ in the evidence they provide for pre-training prediction.

Table 2. Evidence map of representative computing and closed-loop studies relevant to pre-training prediction

Study or platform	Biological system / context	Principal contribution	Relevance to pre-training prediction	Evidence boundary
Brainoware [12]	Brain-organoid computing system	Used organoid dynamics as a biological reservoir for temporal transformation and external readout.	No pre-training electrophysiological predictor of later learning was established in this review.	Its computational use does not show that the organoid learned autonomously.
DishBrain [41]	Human and mouse neural cultures; two-dimensional	Reported task-related adaptation in embodied two-dimensional neural cultures.	Adjacent biological evidence; it does not provide direct pre-training prediction evidence from brain organoids.	A related two-dimensional model, not direct evidence from a brain organoid.
Cortical-organoid cartpole system [15]	Mouse cortical organoids	Reported feedback-dependent improvement and a within-study pre-task causal-connectivity signal.	Direct task-linked organoid evidence: causal connectivity measured before the task was associated with later performance.	One mouse-organoid task and pipeline; no locked predictor was tested in an external dataset.
Supervised biological neural network [45]	Biological neural network; two-dimensional	Demonstrated online learning of temporal patterns under feedback control.	Adjacent biological evidence; no direct pre-training organoid predictor was established in this review.	The biological network was two-dimensional and was not an organoid.
Neuroplatform [13]	Wetware-computing infrastructure	Enabled remote access, longitudinal recording, stimulation, and wetware experimentation.	Enables longitudinal measurement, but prospective prediction before training was not established by the platform itself.	The infrastructure alone does not demonstrate reproducible learning.
Neural organoid microphysiological system [16]	Neural organoid system	Reported connectivity, criticality-related dynamics, and stimulation-induced plasticity.	Organoid mechanistic/supporting evidence; connectivity and dynamics were measurable, but prospective task-performance prediction was not tested.	Supports a plausible mechanism, but not prospective prediction of task performance.
Multi-organoid loop cerebral connectoids [60]	Three or four human cerebral organoids connected through axon bundles	Showed richer temporal dynamics and sequence-specific entrainment.	Organoid mechanistic/supporting evidence; multidomain dynamics were measurable, but prediction of later task learning was not tested.	Shows complex network dynamics and stimulus-dependent modulation, but not task learning or prospective prediction.

Controls on the digital side are needed before performance can be assigned to the organoid. A decoder can improve even when it receives the same fixed recording, and apparent gains may also come from normalization, channel selection, or repeated tuning on the test set. Useful comparisons include the real organoid signal, time- or channel-shuffled versions, noise-matched inputs, fixed recordings paired with a trainable decoder, and a suitable digital reservoir. Blocking a proposed plasticity mechanism pharmacologically can provide additional biological support, but it does not replace these computational controls.

Usually, no single control is enough to attribute a gain to the biological system. The observed improvement may contain contributions from maturation, interface drift, nonspecific stimulation, behavior-linked feedback, decoder training, and biological adaptation. Each control rules out a different part of this mixture. Investigators should begin by defining the estimand, meaning the exact effect to be estimated, and then select controls that address it.

3. What to measure before training

Before training, the practical question is whether the organoid-interface system produces a signal that is usable, informative, responsive, and stable enough for the planned task. Figure 2 follows the path from the biological sample and recording interface to spontaneous network activity, a small probe response, and the final task decision. A failure at any stage can prevent a reliable task decision.

This is a measurement sequence, not a claim that biology follows a fixed hierarchy.

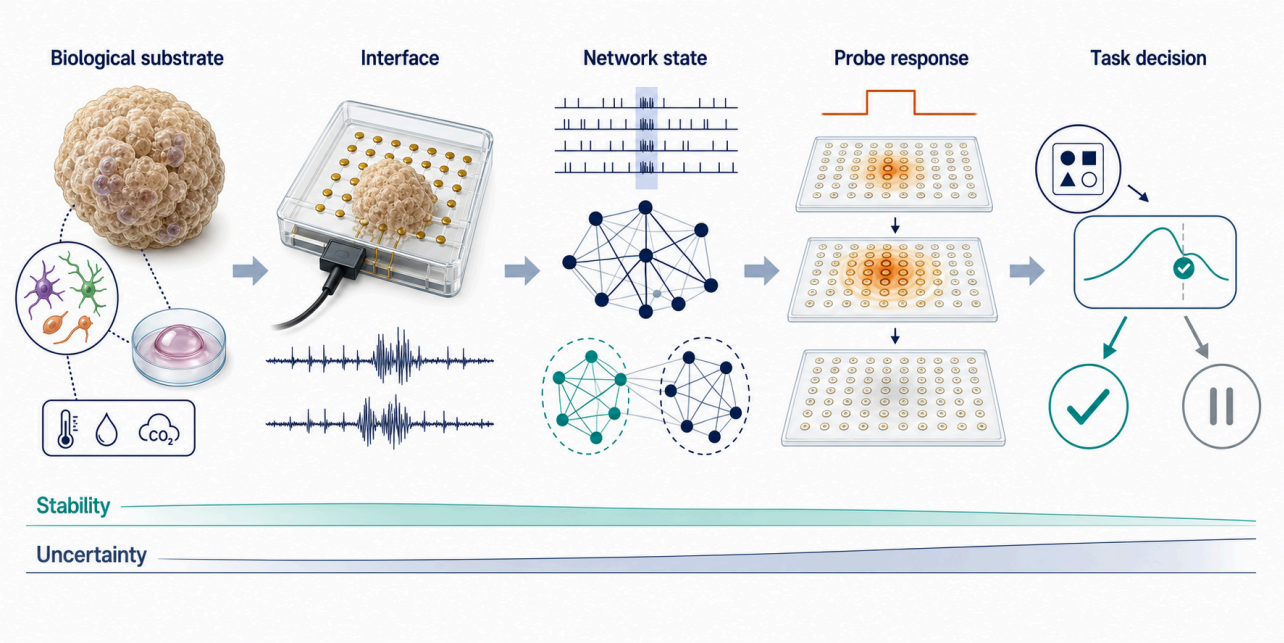


Figure 2. From the organoid and recording interface to a task-specific decision.

3.1 *Is the recorded signal usable?*

Mean firing rate, active-electrode proportion, spike amplitude, interspike intervals, and burst frequency offer a basic check of whether neural activity can be measured reliably [2,51]. Sparse activity can make responses difficult to detect. At the other extreme, high activity may indicate general excitation or excessive synchrony rather than a better recording. The relation between baseline activity and later performance is therefore unlikely to be linear. These measures should be read with signal-to-noise ratio, channel loss, artifact rate, and repeated baseline recordings.

Activity alone is a weak predictor. A high firing rate does not show that the network can distinguish inputs or retain information. In practice, activity measures may be most useful for excluding recordings that are too weak or unstable for further analysis. Thresholds will remain platform specific until cross-platform calibration is available.

3.2 *Does the activity have useful timing?*

Network bursts can be described by their frequency, duration, participating channels, spatial spread, and intervals. These features show how the network moves between coordinated states, whereas a single burst rate discards much of the timing information. Burst sequences, state transitions, autocorrelation, and recovery may therefore be more informative.

Neuronal timescales describe how long earlier activity continues to influence the network [32]. Such persistence may support fading memory, although the useful range is task dependent. If the timescale is too short, relevant history may be lost; if it is too long, the network may respond less readily to new input.

3.3 *Are network states both coordinated and distinguishable?*

Some coordination is needed for computation, but complete synchronization would leave little room for distinct network states. An intermediate regime may allow communication across the network without erasing state differences. Pairwise correlation, spike-time tiling coefficient, phase locking, burst participation, and frequency-specific coherence measure different aspects of coordination, so their values are not interchangeable. Broad, global synchrony may reduce the number of states available to a task.

Oscillatory activity in organoids changes with development [6,8]. Even so, spectral power depends strongly on the reference, filtering choices, and artifacts. Frequency relationships and brief oscillatory events should be considered alongside spatial propagation and repeatability. A spectrum described as "brain-like" does not by itself demonstrate equivalent cognition.

For reservoir computation, nonlinear state changes must also recur reliably. Entropy, dimensionality, participation ratio, recurrence, state-space coverage, and stimulus-response separation describe different parts of this behavior, but a larger value is not always preferable. Noise and shifting electrode contact can both inflate apparent complexity without improving repeatable decoding. Criticality-related measures address a narrower question: is activity near a transition between ordered and disordered states, where responsiveness and dynamic range may increase [16,43]? The answer is sensitive to sampling, avalanche definitions, time-bin selection, and the assumptions of the model. Intelligence does not require criticality, and a critical state alone is not evidence of intelligence. Its practical value here would have to be shown by better prediction than simpler features in held-out organoids.

These connectivity measures quantify relationships among recorded signals; they do not necessarily map physical connections or causal influence. Correlation, mutual information, transfer entropy, coherence, and model-based effective connectivity each rest on different assumptions. Estimates of network density, modularity, participation coefficient, and hub structure may be informative, but short recordings or unequal firing rates can make them unstable. Baseline spike count can also make connectivity appear

stronger even when network organization has not changed.

Robbins and colleagues provide the only direct pre-task, task-linked result in this feature family: baseline functional and causal connectivity were associated with later performance [15]. This finding supports further testing, not a validated biomarker. Future studies should prespecify the estimator, use null models and uncertainty intervals, and test whether separate baseline segments give comparable results before evaluating new organoids.

3.4 *Does the organoid respond reliably to a simple probe?*

Spontaneous recordings do not show whether an organoid responds consistently to input. A small standardized probe can measure response probability, latency, amplitude, spatial spread, recovery, trial consistency, and discrimination between input patterns. Paired-pulse, patterned, ionic, or neurotransmitter stimulation may also indicate adaptation or plasticity potential [16,33,39,57].

The probe must not become an unreported training session. The protocol should use a limited input set, define washout periods, and record recovery so that it measures potential while changing the network as little as possible.

3.5 *Is the pattern stable enough to trust?*

Baseline drift, test-retest correlation, coefficient of variation, electrode dropout, and sudden change points can be predictors as well as quality checks. Complex activity has little practical value if it changes unpredictably during a task, but a perfectly stable system with very few states may also have limited adaptability. The relevant question is therefore whether stability and plasticity are balanced for the intended task.

Stability should be checked at more than one timescale: within and between sessions, during stimulation, and after recovery. Repeated measurements provide the baseline needed to distinguish later learning from natural change and show whether a short recording represents the organoid's usual state.

Recent studies show that several electrophysiological properties can be measured together, although they do not yet show that the combination predicts computational learning. Multi-organoid connectoids showed richer temporal activity and sequence-specific entrainment [60]. Dissociated cerebral organoids from six cell lines developed greater connectivity and plasticity but also varied across lines and batches [61]. Patient-derived organoids from 11 individuals with autism spectrum disorder and four controls also differed in baseline activity, evoked responses, plasticity, and network dynamics [62]. These results support multidimensional measurement, not prospective prediction.

Table 3 summarizes the candidate measurements and one limitation of each. None is yet an established predictive biomarker.

Table 3. Candidate pre-training measurements and their principal limitations

Domain	Candidate measurements	Computational rationale and principal caveat	Evidence status
Activity viability	Firing rate, active-electrode ratio, spike amplitude, inter-spike intervals	Establishes usable signal; high activity alone does not indicate information processing	Organoid supporting evidence; predictive value not established
Temporal organization	Burst rate and structure, autocorrelation, neuronal timescales	May reflect integration and fading memory; strongly dependent on age and window definition	Organoid supporting evidence; predictive value not established
Network coordination	Synchrony, coherence, phase locking, burst participation	Intermediate coordination may balance integration and differentiation; excessive synchrony can reduce state diversity	Organoid supporting evidence; predictive value not established
Dynamical richness	Entropy, dimensionality, participation ratio, recurrence, state separability	Supports nonlinear transformation; noise and unstable contact can create false complexity	Organoid computational/supporting evidence; prospective predictive value not established
Criticality-related state	Avalanche statistics, branching-related measures, dynamic range	May identify a responsive regime; estimator and subsampling sensitivity are substantial	Organoid mechanistic/supporting evidence; task-linked predictive value not established
Connectivity	Correlation, mutual information, effective or causal connectivity, modularity	The only candidate family with direct pre-task, task-linked support in a mouse cortical-organoid study; estimates remain rate-dependent, method-sensitive, and unvalidated as a transferable biomarker	Direct pre-task, task-linked organoid evidence [15]; not externally validated
Probe responsiveness	Latency, amplitude, spread, trial consistency, stimulus discriminability	Tests input transformation before training; the probe itself can alter the network	Organoid mechanistic/supporting evidence; prospective predictive value not established
Stability	Drift, test-retest reliability, channel dropout, change points	Determines whether learned mappings are interpretable; excessive stability may also indicate limited adaptability	Conceptual predictor supported by organoid/interface reliability considerations; predictive value not established

3.6 *Putting the measurements together for a specific task*

Current organoid quality control focuses mainly on shape, identity, cell composition, differentiation, and reproducibility [21-25]. A recent preprint extends this discussion toward rational quality-control design [63]. Nondestructive imaging, morphology-based selection, and Raman measurements may allow repeated assessment with little damage [30,34,64]. Functional screening adds a narrower question: can this organoid produce stable and informative activity for the planned task?

A simple summary can ask six questions: Is the signal usable? Does activity have useful timing? The other questions concern coordination between distinguishable network states, response to a probe, plasticity of that response, and measurement stability. They should be answered separately because a sample can perform well in one domain and poorly in another. For example, an organoid may give a strong response yet

drift across sessions; another may be stable but produce too few distinct states for the intended task.

Combining all six answers into one score would hide these trade-offs. A measure useful for classification may be less relevant to temporal prediction or closed-loop control, and a fixed threshold may fail on another platform. Standardized values can assist comparison, but raw measurements, uncertainty intervals, and missing-data labels should remain visible. The cost of including an unsuitable organoid differs from the cost of excluding a suitable one, so thresholds should be reconsidered when the task, platform, or biological source changes.

A measurement should be treated as a predictor only after three checks. The first is construct validity: does the measure change as expected with maturation, coupling, activity, or plasticity? Reliability asks whether repeated measurements agree under stable conditions. Predictive validity asks whether the measurement forecasts an independently defined outcome in organoids that were not used to develop the model. The model should withhold a prediction when activity is insufficient, the interface shifts, drift is extreme, stimulation may have caused damage, or the sample lies outside the development data. Biological absence, technical failure, and an uncollected measurement should be recorded separately rather than treated as the same low value.

4. Turning pre-training measurements into a prediction

Once the pre-training measurements have been defined, the next question is whether they predict a future task outcome. The outcome, time horizon, task, and intended decision must be fixed before model development. Otherwise, the same data can be searched for whichever definition of learning produces the strongest association. Figure 3 summarizes this workflow, linking pre-training measurements to a prespecified outcome, task matching, grouped model development and validation, and decision utility.

4.1 *Define the outcome and prediction horizon*

A predictive biomarker is meaningful only when its outcome is defined before analysis. "Learning ability" could refer to task success, final performance, change from baseline, learning-curve slope, time to a threshold, retention after rest, resistance to noise, or generalization to new inputs. These outcomes are related but not equivalent. Final performance partly reflects where an organoid began, whereas change from baseline places more weight on adaptation. Time to threshold measures efficiency only when the threshold has a clear justification. Retention and generalization provide stronger tests, although both require extra experiments.

Primary outcome selection should follow the intended use. For sample selection, a model might estimate whether an organoid will reach a minimum performance level within a specified training period. A biological study may instead focus on the learning trajectory and retention after training. For comparisons between platforms, change relative to a matched control is usually easier to interpret than raw accuracy. Other outcomes can remain secondary, but the primary outcome should be fixed before the model is developed.

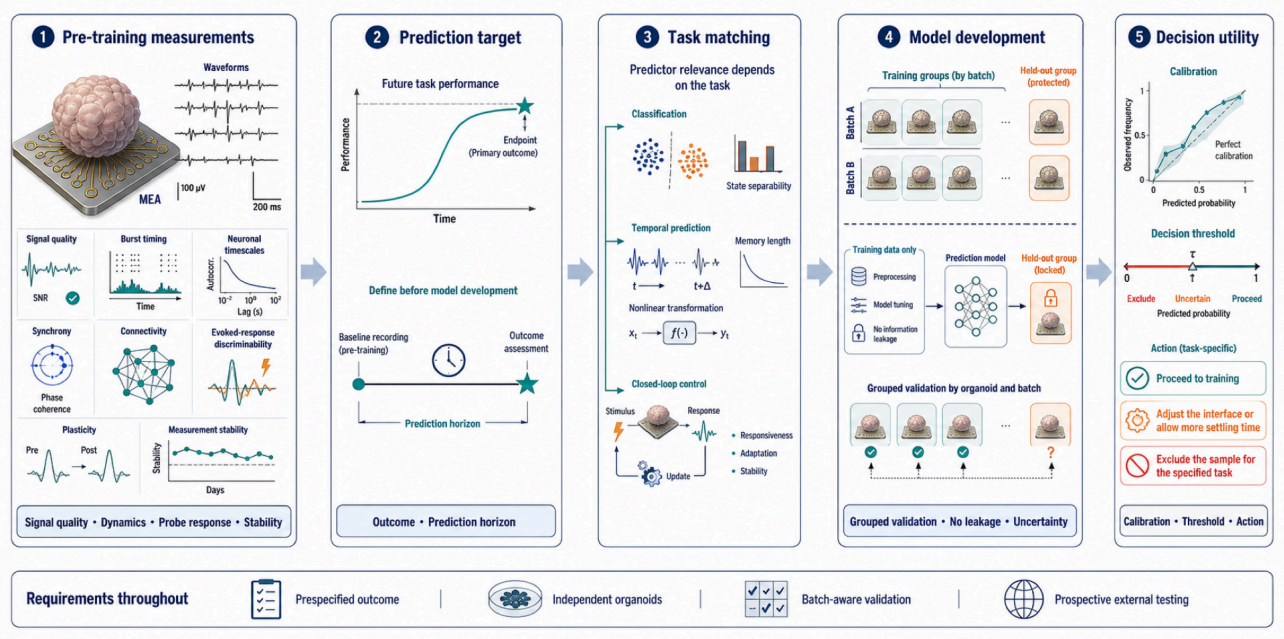


Figure 3. Workflow for translating pre-training measurements into task-specific prediction and decision-making.

The interval from baseline recording to the outcome defines the prediction horizon and should be chosen before the results are known. A recording made just before training mostly describes the organoid's current state and its present interface coupling. A recording from several days earlier may be more useful for production planning, provided that its relation to the outcome survives maturation and interface drift. Researchers could prespecify several intervals to ask whether a feature reflects stable capacity or only short-term readiness. Choosing the most favorable interval after examining the outcomes would overstate predictive performance.

4.2 *Relate predictors to the computational task*

Performance on one task cannot be taken as general computing ability. A classification task mainly tests whether network states can be separated. Temporal prediction adds requirements for memory and nonlinear transformation. Closed-loop control also depends on responsiveness, adaptation, and stability during repeated feedback. The association between an electrophysiological feature and performance may therefore change with the task.

Across tasks, a predictor set should describe stable properties of the organoid rather than restate one benchmark score. Candidate dimensions include temporal memory, nonlinear transformation, input separability, adaptability, resistance to disturbance, and stability. One test is to have the same organoids perform several standardized tasks. If this is impractical, closely related organoids from one biological source can be distributed across tasks. Either design must balance task order because an earlier task may alter later performance.

Task-general does not have to mean one ranking. An organoid with long temporal memory may have only moderate input separability, while another may adapt quickly but remain unstable. A task-matched summary should keep these trade-offs visible and may reasonably se-

lect different organoids for different computational uses.

Multi-task models can separate predictors shared across tasks from those that matter only for one task. Leave-one-task-out testing asks whether measurements learned from several tasks transfer to a new one, while holding out a batch or cell line tests biological transfer. A feature that fails these tests may still be useful within one protocol, but it is not task general.

For practical use, matching an organoid to a task may be more useful than ranking every organoid from best to worst. This remains a proposal for prospective testing, not a validated application.

4.3 *Build a predictor that the biological sample can support*

With only a small number of independent organoids, it is generally safer to begin with physiologically grounded features chosen in advance. Penalized regression, partial pooling, and Bayesian models can reduce overfitting while keeping uncertainty visible. Random forests and gradient boosting can model nonlinear relations, but their complexity must be supported by the number of organoids, not by the much larger number of windows or electrodes. Deep representation learning is difficult to justify unless the number of independent biological samples can support it. A pretrained representation offers another option, but its evaluation must use only organoids that played no part in model development.

Every resampling loop must rebuild the complete feature pipeline. If normalization, channel selection, missing-value imputation, dimension reduction, or hyperparameter tuning occurs before the train-test split, information from the test data can enter model development [65]. Fit each operation using the training groups, then apply it unchanged to the held-out group without further fitting. Reports also need to document how raw voltage became the final prediction and which information was available when each analytical choice was made [66].

4.4 *Does the model lead to better decisions?*

Correct ranking alone does not make a screening model useful. Predicted probabilities may still disagree with observed outcome frequencies even when the ordering looks reasonable. Evaluation should examine calibration plots, calibration intercept and slope, Brier score, uncertainty intervals, and either AUROC or correlation. A small validation set permits only a coarse calibration assessment; fitting a flexible curve can create a false impression of precision [67]. Any screening threshold also has two consequences: resources may be spent on an organoid that is not ready, while a potentially successful organoid may be rejected. The preferred balance will depend on organoid availability, task cost, and the acceptable failure rate.

An interpretation has practical value when it leads to a specific response. If unstable baseline activity precedes failure, researchers could adjust the interface or allow more settling time. Poor discrimination between evoked responses may instead point to the input encoding or the placement of stimulation. Identifying a limitation that can be addressed is more useful than assigning a score without explanation.

5. Distinguishing biological learning from apparent improvement

5.1 *Sources of apparent improvement*

Living neural tissue changes even when it is not being trained. Maturation, metabolism, medium exchange, temperature, oxygen, cell death, network reorganization, and shifting electrode contact can all change the recorded activity. Repeated stimulation introduces habituation, sensitization, fatigue, and nonspecific plasticity as further explanations. Improvement can also arise on the digital side from readout optimization, feature selection, or repeated inspection of test data. A before-and-after comparison, on its own, cannot separate these effects.

The causal question is easier to evaluate when the estimand, or exact effect to be estimated, is written down. A study could ask, for example: among viable organoids using a fixed decoder protocol, what is the average effect of behavior-contingent closed-loop feedback on performance after training compared with matched feedback unrelated to behavior? This question states the treatment, comparison, outcome, population, and digital conditions. It also stops "learning" from being used as a label for several different effects.

5.2 *Counterfactual controls for competing explanations*

Each control rules out a different explanation. A no-stimulation group captures biological change and interface drift over time. Sham or passive stimulation also includes handling and electrical exposure. Random feedback asks whether improvement depends on a link between behavior and feedback. In a yoked-replay condition, one organoid receives the stimulus sequence generated by another but cannot affect what comes next. Both organoids receive the same sequence, but only one participates in a true closed loop. Digital-only and shuffled-signal baselines then estimate how much performance remains when the biological response's real timing and channel structure are removed.

Table 4 shows which effect each control helps identify. An experiment may not need all of them, but its learning claim cannot be stronger than the alternative explanations excluded by its design.

Because these controls answer different questions, the minimum set depends on the claim. Feedback-dependent adaptation requires a comparison in which feedback is not related to behavior. A claim of biological computation also needs digital baselines and shuffled biological signals. Retention is a separate question and should be tested after training, when the decoder is no longer being fitted.

5.3 *Analyze longitudinal and hierarchical change*

Reducing repeated performance and electrophysiological measurements to a before-and-after comparison discards much of the available information. Mixed-effects models can retain the structure of trials within organoids and organoids within batches, while flexible time terms can capture nonlinear learning or fatigue. Another approach is a state-space model, which separates a latent biological state from measurement noise. Change-point methods may help locate abrupt changes in either the network or the interface. Whatever method is used, permutation tests must keep related observations together; shuffling individual time windows across organoids breaks that dependence and creates an invalid comparison.

Table 4. Counterfactual controls for evaluating learning claims

Control category	Control condition	Component estimated	Interpretation enabled
Biological / time	No stimulation	Maturation, culture change, recording and interface drift	Whether time-dependent change occurs without task exposure
Stimulation exposure	Sham or passive stimulation	Handling and nonspecific stimulation exposure	Whether repeated input alone changes the outcome
Feedback contingency	Random feedback	Feedback exposure without meaningful contingency	Whether behavior-related consequences are required
Feedback contingency	Yoked replay	Identical stimulus sequence without control of future input	Whether closed-loop contingency adds an effect beyond exposure
Digital / computational	Shuffled biological signal	Loss of true temporal or channel structure	Whether decoding depends on organized organoid dynamics
Digital / computational	Fixed recording with trainable decoder	Digital readout optimization	How much improvement occurs without biological adaptation
Digital / computational	Digital reservoir or digital-only model	Nonbiological computational baseline	Whether the organoid adds task-relevant value under a fair pipeline

Consider a treatment-by-time interaction. This interaction estimates whether the closed-loop group changed more than the control group. Even so, the contrast is not causal by default, because its interpretation still depends on parallel trends and the model's other assumptions. Researchers should therefore examine whether the result survives alternative time windows, exclusion rules, definitions of task success, and decoder-freezing procedures. With only a few organoids, a Bayesian hierarchical model can borrow information across groups while retaining uncertainty at the organoid and population levels.

5.4 *Disentangle biological and digital adaptation*

Some experimental designs allow the organoid and decoder to change at the same time. This joint change is not automatically a flaw, but it answers a different question. A high-performing biohybrid system may benefit from co-adaptation. A study intended to demonstrate biological learning, however, must freeze decoder updates during the main evaluation or separate them through the experimental design. The protocol should state which part of the system can change during each phase.

Figure 4 maps the main sources of apparent improvement to the controls that can separate them.

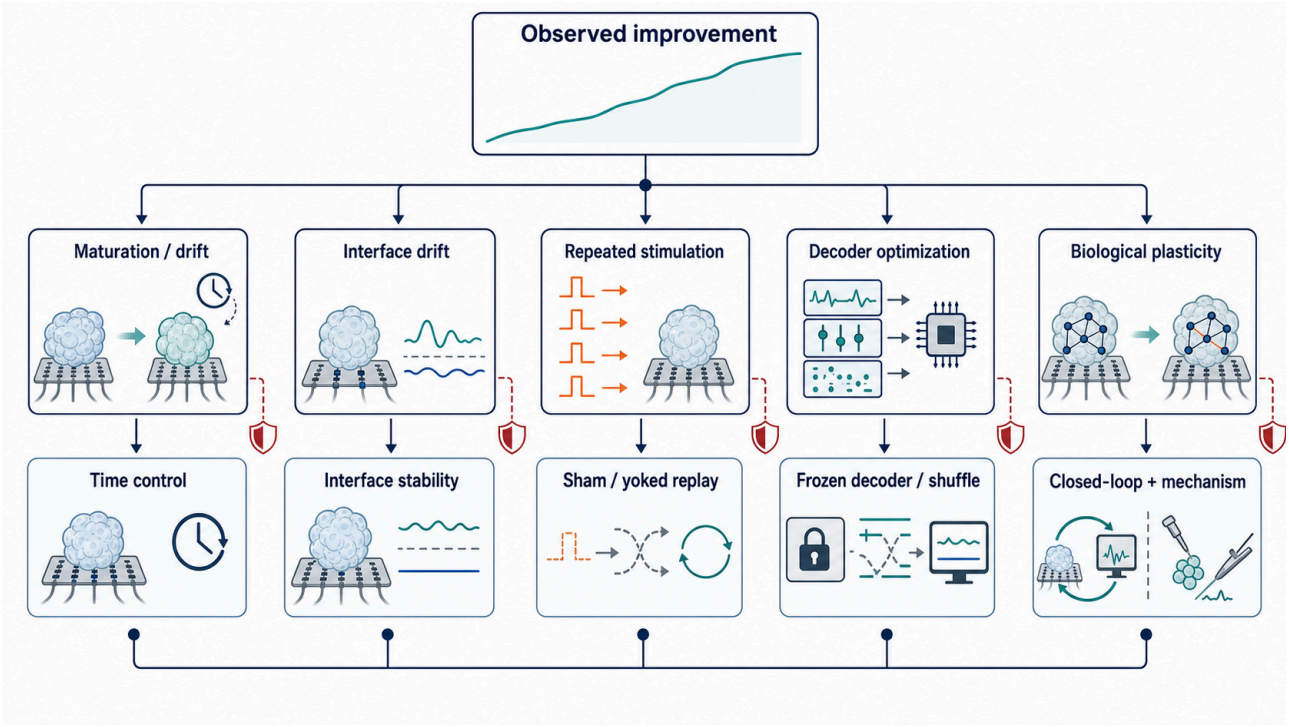


Figure 4. Sources of apparent task improvement and the controls used to distinguish them.

6. Validating predictors for functional quality control

Biological plausibility alone does not make a measurement a valid predictor. A credible evaluation also needs a traceable data pipeline, independent biological test units, model complexity suited to the sample size, and explicit treatment of batch effects, missingness, and transfer.

6.1 *Make the data pipeline traceable*

Filtering, artifact removal, spike and burst definitions, referencing, window length, and active-channel criteria can all change the resulting electrophysiological features. Reproducibility therefore depends on tracing the route from raw voltage to the final predictor. The record must also specify the code, parameter settings, exclusion rules, units, mathematical definitions, and software versions.

Learned representations require the same caution. A latent feature may primarily identify the batch, platform, or individual organoid rather than computational capacity. These possibilities need to be tested before the feature receives a biological interpretation.

6.2 *Keep validation free of data leakage*

All measurements from one organoid must remain in the same validation fold. Windows and electrodes share a biological and technical history; splitting them across training and test sets lets a model recognize an organoid it has already seen. This is pseudoreplication [68]. When possible, studies should also hold out complete batches and later test new cell lines, platforms, and laboratories.

Model selection must also be separated from performance estimation. Nested cross-validation reduces optimism when tuning is required [69],

while repeated grouped resampling shows how unstable results may be in small datasets [70]. Sample-size planning should follow the outcome, model complexity, and required calibration precision rather than a fixed rule [71-72]. A prospective or untouched test group remains stronger than repeated internal resampling.

Figure 5 links the biological hierarchy used for leakage-free validation with the stages required for translation to functional quality control.

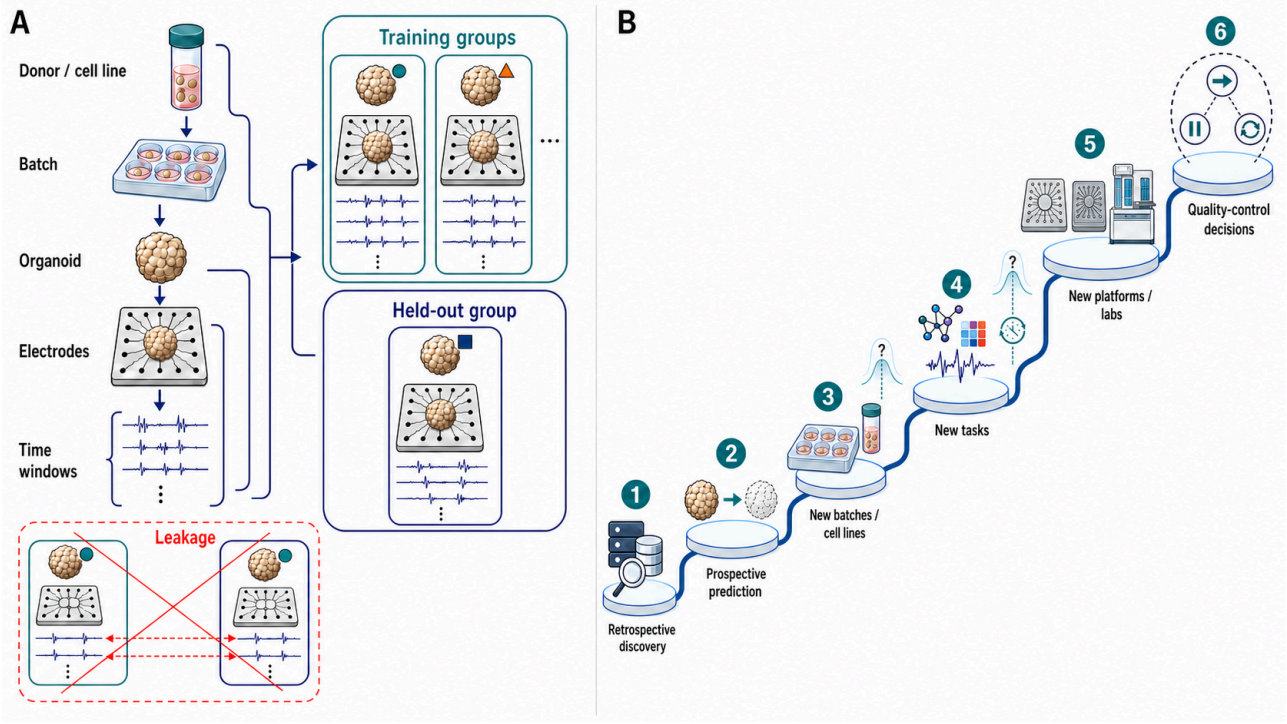


Figure 5. Leakage-free validation and staged translation to functional quality control.

6.3 *Match model complexity to the available organoids*

Electrophysiology measures function but does not fully explain the biology producing the signal. Live imaging can follow morphology and development without destroying the organoid [30,34], while transcriptomic or proteomic data can describe maturation and cell composition. These additional modalities can help determine why organoids with similar electrical activity do not always behave alike.

The relation between modalities should be reported rather than assumed. Authors need to specify whether each measurement came from the same organoid, a different organoid in the batch, or an external reference, and whether the collection times coincided. When sample coverage or missingness differs across modalities, late fusion or hierarchical models are preferable to an analysis that treats every observation as perfectly matched.

Spikes do not determine the effective sample size; independent organoids do. A large feature set built from only a few organoids is prone to overfitting and unstable feature selection [65,70]. A safer starting point is a small, prespecified set with shrinkage or a simple baseline.

Feature selection and dimension reduction must then be repeated inside each resampling split.

6.4 *Include batch effects, failures, and uncertainty in validation*

Validation data should include batch variation, organoid failures, and missing channels. Removing these cases without explanation produces an unrealistically clean sample and inflates apparent reliability. Domain shift across cell lines, media, protocols, interfaces, and laboratories should be expected. Each new setting therefore requires fresh checks of performance and calibration. Recalibration alone is appropriate only when the predictor-outcome relation has otherwise remained stable.

Reports should give the numbers of donors, cell lines, differentiations, batches, organoids, electrodes, sessions, and windows, together with their nesting and every exclusion. They should also describe the data split, preprocessing, primary outcome, missing-data plan, calibration, uncertainty, and biological and digital controls. Thousands of windows cannot replace missing information about organoids or batches.

Several defensible preprocessing choices may produce different feature matrices. Sensitivity analyses should compare whether organoid rankings, calibration, and conclusions change across these choices. When conclusions depend on one narrow pipeline, that uncertainty should be reported.

Table 5 summarizes the main statistical failure modes and the minimum response to each.

6.5 *Work within current experimental limits*

Current interfaces sample only part of a three-dimensional organoid and are often most sensitive near the surface [20,51]. Mesh, embedded, multilayer, and high-density arrays increase coverage or depth access [52-55], but contact can shift as tissue grows. Culture time, necrosis, maturation, stimulation artifacts, and platform capacity further limit throughput. Automated maintenance, acoustofluidic culture, repeated imaging, remote platforms, and longitudinal unit tracking may improve continuity [13-14,34,38,56].

Even with these limits, current platforms can record spontaneous and evoked activity for weeks, sample several depths or most of the surface, and deliver spatially structured stimulation [16,55,58-60]. They can quantify connectivity, network activity, and plasticity-related features [61,62]. Mouse cortical organoids have improved on a closed-loop task, and paired human organoids have shown related results in a preprint [15,17]. However, no frozen multifeature model has yet produced calibrated predictions in independent human organoids, and retention was limited in the strongest peer-reviewed task study [15].

AI may help analyze longitudinal recordings, select informative probes, or optimize culture and stimulation conditions, but the objective must be fixed in advance. Optimizing firing rate or decoder accuracy alone may reward instability or digital shortcuts. Standards should focus on measurable requirements such as noise, coverage, artifacts, latency, coupling stability, and repeatability while allowing different interface designs. Energy comparisons must include the resources used for culture maintenance, operation of the interface, and digital processing.

Table 5. Statistical threats to valid prediction of learnability

Threat group	Problem	Consequence	Minimum corrective action
Data structure	Pseudoreplication	Electrodes, windows, and trials are treated as independent organoids	Preserve the organoid as the biological unit and use hierarchical models where appropriate
Model development	Small samples and high dimensionality	Feature selection becomes unstable and apparent performance is optimistic	Prespecify or reduce features, regularize, and report uncertainty against simple baselines
Biological variation	Batch, donor, and cell-line effects	Models learn production history instead of computational capacity	Use grouped splits, domain-specific reporting, and external biological validation
Biological variation	Culture age and maturation	Natural time trends imitate training or readiness effects	Age-match controls and model time trends explicitly
Measurement / selection	Missingness and failed coupling	Complete-case analysis selects unusually clean organoids	Document each reason, keep failure flags, and assess the missingness mechanism
Model development	Multiple testing	Searching many features, windows, and outcomes creates chance findings	Specify primary predictors and outcomes in advance, and clearly mark exploratory analyses
Model development	Data leakage	Held-out organoid information enters preprocessing or feature selection	Repeat the complete pipeline within grouped nested validation
Evaluation	Calibration and thresholding	Accurate rankings still produce incorrect readiness probabilities or decisions	Report calibration, and choose thresholds without viewing the test set
Evaluation / transfer	Domain shift	New platforms or laboratories alter signal distributions and relationships	Validate by domain, detect out-of-distribution samples, and prespecify recalibration rules
Reporting	Selective reporting	Only successful organoids, windows, or models remain visible	Preregister exclusions and report the complete sample and model flow

6.6 *A staged path to functional quality control*

Phase 1 should use existing data to establish reliable features and outcomes, with every measurement from one organoid kept in the same analysis group. Before Phase 2 begins, investigators freeze the feature pipeline and model, then evaluate them in a preregistered prospective task. The model must be compared with simple baselines based on age, shape, or activity, and its calibration and uncertainty should be reported.

Phase 3 evaluates the model in new batches and cell lines. Phase 4 examines transfer across tasks, and Phase 5 moves to another interface or laboratory while identifying samples outside the training distribution. Only Phase 6 converts validated predictions into functional quality-control thresholds and measures the consequences of incorrect inclusion or exclusion.

Models will need reevaluation when culture or recording methods change. Versioned reference data and prespecified recalibration rules can keep the process traceable. Practical value

should be judged by recording burden, failed measurements, avoided training attempts, and incorrect exclusions. A negative result is still useful because it may show that baseline electrophysiology contains too little predictive information or that task performance depends mainly on the interface or decoder.

Table 6 summarizes the six phases and the evidence required before functional quality control.

Table 6. Staged validation roadmap from retrospective discovery to functional quality control

Evidence status	Phase	Main test	Minimum deliverable
Exploratory / retrospective	1. Retrospective discovery	Identify reliable candidate features and outcomes	Locked feature definitions, grouped resampling, simple baseline comparison
Prospective validation	2. Prospective prediction	Predict learning before task exposure	Frozen model, preregistered primary outcome, calibration and uncertainty
Prospective validation	3. Biological generalization	Test new batches and cell lines	Leave-one-batch/cell-line-out performance and recalibration analysis
Prospective validation	4. Task generalization	Test whether predictors transfer across computational demands	Shared and task-specific phenotype estimates
Prospective validation	5. Platform and laboratory transfer	Test new interfaces and sites	External validation plus out-of-distribution detection
Prospective validation	6. Functional QC implementation	Convert prediction into sample-selection decisions	Thresholds linked to cost, false exclusion, failure rate, and monitoring

7. Conclusions and future directions

7.1 *Conceptual and ethical boundaries*

Computing, learning, intelligence, sentience, and consciousness refer to different claims. The evidence reviewed here concerns measurable neural dynamics, plasticity, and performance on limited tasks. Human-like language can overstate both the scientific finding and the ethical concern, so working definitions should remain tied to the demonstrated level of function. The proposed pre-training measurements do not assess consciousness, sentience, perception, subjective experience, or moral status. They address features related to performance under a defined task and interface.

OI also raises questions about donor consent, control of electrophysiological and molecular data, commercial use, experimental endpoints, and the treatment of increasingly complex neural models [9,73-74]. Patient- or donor-derived organoids may contain genomic and health-related information. Governance therefore needs to cover access, limits of de-identification, secondary use, data linkage and sharing, commercial use, and unexpected findings. When adaptive biocomputing extends well beyond ordinary disease modeling, consent documents should distinguish those uses. Ethical review should become more demanding as functional complexity and evidence increase, while avoiding the opposite error of treating electrical activity alone as evidence of consciousness.

7.2 *Shared data, benchmarks, and cross-laboratory testing*

The field needs a shared measurement system that links biological state, interface quality, task

design, and computational outcome more urgently than it needs additional games for organoids. Shared datasets should contain raw electrophysiology, standard metadata, repeated baselines, stimulation logs, task trajectories, failed cases, and batch identifiers. A useful benchmark would define controls and independent validation units rather than report accuracy alone. Hidden test labels, group-aware evaluation, versioned preprocessing, and challenge datasets from new laboratories would test whether a model works beyond the dataset in which it was developed.

Self-supervised models may learn reusable neural states from long recordings, and multimodal models may connect those states with imaging and molecular data. Digital twins or virtual-organoid models could then help design experiments, but they would need repeated longitudinal updates and prospective validation. Within such a system, active learning could select the next experiment that reduces the greatest uncertainty about readiness or biological mechanism.

Strong performance inside one dataset cannot establish that a model will work elsewhere, so cross-laboratory studies will be necessary. Shared reference organoids, calibration signals, preprocessing benchmarks, and reporting standards may help separate biological properties from platform-specific measurements. The strongest evidence for computational learnability would combine a prediction specified in advance, a task design that addresses alternative explanations, support for a biological mechanism, and replication outside the original study.

7.3 *Readiness depends on the intended use*

Readiness has a different meaning when the organoid is intended to model disease. Hyperexcitability or unusual timing may lower performance on an engineering benchmark while still providing an important clinical phenotype or greater sensitivity to a drug or toxic substance [75-78]. Functional readiness should not be read as a general measure of biological health. Its outcome and threshold should be set for the intended use, whether engineering, disease modeling, toxicity testing, or developmental comparison.

7.4 *Conclusion*

Brain organoids can produce organized activity, plastic responses, and dynamics that support proof-of-concept computational systems. The question now extends beyond whether living neural tissue can generate a useful output: can researchers predict, explain, and reproduce improvement on a defined task before deciding to train a particular organoid?

Pre-training electrophysiology is a plausible starting point, although the evidence remains narrow. In a study of mouse cortical organoids, connectivity measured before the task was associated with later performance [15]. Researchers have not yet tested a multifeature profile across independent human organoids, batches, tasks, interfaces, and laboratories. A credible predictor would need to integrate the biological substrate, interface quality, spontaneous network state, probe response, and task-specific outcome without hiding the associated uncertainty.

Rather than proposing a universal score, this review identifies a practical set of measurements for testing. The proposed measurements must predict prespecified outcomes in biological units withheld from model development. Experiments must then distinguish biological learning from natural drift, repeated stimulation, and changes to the decoder. Prospective success on both re-

quirements would justify testing functional quality control as a way to select an organoid for a particular task. For now, the evidence supports that research program, not routine screening or a threshold shared across systems.

Author contributions

Yin Pui Wang conceived the review, evaluated and synthesized the evidence, wrote the manuscript, and prepared the visualizations. He also led the revisions and language editing. Mingliang You supervised the project and provided critical review.

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Competing Interests

Yin Pui Wang completed this review during an internship at Hangzhou AimingMed Technologies Co., Ltd. The relationship is disclosed because the review addresses functional quality control in an industrial setting.

Data Availability

No original dataset was created for this review.

Use of Generative Artificial Intelligence

Generative AI tools assisted with language editing, manuscript organization, and the initial development of figures. Yin Pui Wang subsequently reviewed the full text, checked the scientific statements against the cited sources, and takes responsibility for the final manuscript.

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