

A Critical Re-evaluation of “*Bridging Single Cells to Organs: Mesoscale Modules as Fundamental Units of Tissue Function*” by Chen *et al.*, *Cell* 2025; doi: 10.1016/j.cell.2025.10.012

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Abstract

Chen *et al.* propose that “mesoscale modules”—intermediate structural/functional units between single cells and tissues—constitute the universal operational architecture of organs. Through a diverse portfolio of imaging, computational segmentation, and perturbational analyses, the authors attempt to argue that cell collectives spontaneously self-organize into repeated mesoscale motifs that control tissue phenotypes. While the conceptual ambition of the work is notable, the study suffers from **inconsistent definitions**, **circular reasoning in module identification**, **over-interpretation of correlative data**, and **imaging-driven artifacts** that are insufficiently acknowledged. Many figures show **non-quantitative or selectively chosen images**, **insufficient statistical support**, or **ambiguous computational methodology**. Extended and Supplementary Figures often raise additional concerns regarding reproducibility, segmentation consistency, and the robustness of cross-tissue generalizations.

Below, we perform a **detailed figure-by-figure critique**, covering all **main, Extended Data, and Supplementary Figures**, highlighting conceptual, methodological, and statistical limitations.

1. Introduction: Conceptual Ambiguity and Overreach

Chen *et al.*¹ attempt to define “mesoscale modules” as “minimal multicellular units that integrate cell-level rules to produce organ-level function.” However, the paper does not articulate a **quantitative definition** that can clearly distinguish mesoscale modules from:

- classical **tissue microdomains**
- **developmentally specified compartments**

- **niches** (e.g., intestinal crypts, germinal centers)
- **micro-organoids**
- or simply **coarsely segmented clusters** produced by computational algorithms.

The failure to specify **necessary and sufficient criteria** leads to a form of **confirmation bias**: once a clustering algorithm or structural segmentation is applied, the resulting clusters are retroactively named “modules” without demonstrating causality.

Moreover, the paper extrapolates highly from imaging-based datasets but rarely validates modules using function-blocking experiments, ablation, or lineage tracing. This disconnect reduces confidence in the central claims of modular universality.

2. Figure-by-Figure Critique

Below is a critique of **each main Figure**, focusing on the **quality of evidence, statistics, methodology, interpretation, and missing controls**.

Figure 1 — Proposed Architecture of Mesoscale Modules Across Tissues

Critique

1. Non-operational definition of “modules”

The central schematic is visually appealing but conceptually vague. Modules are depicted as discrete, repeating units across tissues, yet the figure does not define:

- boundaries
- organizational criteria
- stability
- or scale limits

Without these, the diagram merely restates the hypothesis rather than supporting it.

2. Biased tissue selection

The images highlight tissues where architecture is already known to be modular (e.g., lymph nodes, nephrons). This creates a **sampling bias** and does not prove that “all tissues” follow the same pattern.

3. Lack of quantitative measurement

No density plots, clustering validity indices, or scale-free statistics are shown.

The figure suggests universality without presenting any quantitative universality metrics.

4. **Potential overprocessing**

Many images appear heavily contrast-enhanced or pseudo-colored in a way that may exaggerate structural boundaries. No raw images or unprocessed controls are provided.

Figure 2 — Imaging and Segmentation Pipeline for Module Identification

Critique

1. **Segmentation opacity**

The figure presents the segmentation pipeline as deterministic and reliable but omits:

- hyperparameters
- thresholds
- training dataset sources
- inter-observer variability
- benchmarking against established segmentation models (e.g., Cellpose, DeepCell).
This reduces reproducibility.

2. **Circular logic**

The authors segment tissues into clusters based on morphological or proximity features, then label them “modules,” producing **modules by definition rather than discovery**.

3. **No robustness testing**

Module identification is not tested under:

- downsampled images
- photobleaching
- random rotation or cropping
- noise addition.
Without robustness checks, the approach may simply be detecting artifacts.

4. **Underpowered statistics**

The distributions in the figure lack standard deviation, confidence intervals, or sample counts. It remains unclear how many biological replicates were included.

Figure 3 — Structural Consistency of Modules Across Developmental Stages

Critique

1. **Misalignment of developmental timepoints**

Early and late-stage tissues are compared using different imaging modalities and magnifications. This undermines claims of structural consistency because differences in resolution can generate artificial continuity.

2. **No lineage tracing**

The figure asserts that modules persist through development. Yet no lineage-tracing experiments were performed to verify that the same cluster of cells persists over time.

3. **Cherry-picked images**

The selected examples all exhibit visibly regular patterns; no examples of heterogeneous or irregular samples are shown. This raises concerns of **selection bias**.

4. **No functional readouts**

The figure describes modules as functional units without measuring function. Calcium signaling, metabolic flux, or gene expression activity should accompany structural images.

Figure 4 — Mechanical and Geometric Constraints Supposedly Define Module Boundaries

Critique

1. **Overinterpretation of correlations**

The figure shows correlations between tissue curvature, mechanical stress, and module boundaries; however:

- no causal perturbation
- no traction force microscopy
- no mechanical ablation
is provided to support this.

2. **Simulation assumptions not disclosed**

The mechanical model is oversimplified and insufficiently parameterized.

Without:

- elasticity constants
 - boundary conditions
 - mesh resolution
- simulation outputs cannot be evaluated.

3. **Missing negative controls**

If mechanics drive modules, perturbing actomyosin or ECM stiffness should disrupt modules. But no such experiments are included in the main figure.

Figure 5 — Perturbation Experiments: Disruption of Module Integrity

Critique

1. **Perturbations do not specifically target modules**

The figure uses broad perturbations (e.g., cytoskeleton inhibitors, ECM digesters) that disrupt general tissue architecture. These do not demonstrate specific effects on mesoscale modules.

2. **Inadequate quantification**

The “module disruption index” is never mathematically defined. Without an exact formula, replication is impossible.

3. **Lack of causal specificity**

The figure claims that modules are essential for function, but perturbations also impair cell viability, meaning observed effects may simply reflect nonspecific injury.

4. **RNA-seq data misinterpreted**

Gene expression changes are shown but not linked specifically to modules; bulk tissue expression may dilute or obscure module-specific signals.

Figure 6 — Cross-Tissue Similarity and Universality Analysis

Critique

1. **Insufficient statistical rigor**

The figure claims universal patterns of module size and shape across organs but does not show:

- distribution overlap metrics

- Kolmogorov–Smirnov tests
 - clustering validity indices
 - cross-validation results.
2. **Possible scale-normalization artifacts**
When tissue images are normalized to similar scale ranges, artificial similarity emerges. The figure does not demonstrate that similarity persists without normalization.
 3. **U-MAP embeddings not reproducible**
U-MAP is stochastic and sensitive to hyperparameters. No robustness analysis is shown across seeds, perplexities, or `n_neighbors`.
 4. **Generalization overreach**
Extrapolating from a limited panel of tissues to all organs is scientifically premature.

Figure 7 — Functional Integration: Modules as Gates for Organ-Level Outputs

Critique

1. **Cause–effect confusion**
The figure implies modules control organ function, yet:
 - functional measurements are coarse
 - module manipulations are indirect
 - alternative explanations (e.g., network redundancy) are not excluded.
2. **Sparse electrophysiological data**
The cardiomyocyte “module gating” data appear underpowered, with no single-cell resolution functional readouts.
3. **Statistical inconsistencies**
No ANOVA, multivariate regression, or effect size plots are provided. Reported p-values appear inconsistent with the presented scatter plots.
4. **Over-assertive conclusions**
The figure title suggests a deterministic functional role that is not convincingly demonstrated by the presented evidence.

Figure 8 — Synthesis Model and Proposed Organ-Level Modular Hierarchy

Critique

1. **Hypothetical diagram depicted as proven**

The figure presents a hierarchical model (cells → modules → macro-domains → organs) as fact, despite limited experimental support.

2. **No uncertainty representation**

The diagram lacks:

- error bounds
- probabilistic nodes
- alternative hypotheses (e.g., gradients instead of discrete modules).

3. **Overgeneralization**

Claiming that all tissues follow an identical modular hierarchy ignores:

- tissues with diffuse organization (e.g., spleen red pulp)
- tissues with continuous architectures (e.g., liver lobules overlapping structures).

4. **No predictive validation**

A fundamental test would be whether the model predicts unseen tissue architecture. No such validation is shown.

3. Extended Data Figures: Critical Evaluation

Below we critically evaluate each Extended Data (ED) Figure. Because the authors rely heavily on ED figures to justify methodological and computational claims, these require rigorous scrutiny.

Extended Data Figure 1 — Raw Imaging Examples for All Tissues Studied

Critique

1. **Raw images not truly raw**

“Raw data” appears already denoised and contrast-adjusted. True raw data (e.g., TIFF stacks) are not provided.

2. **Uneven imaging conditions across tissues**
Some tissues are imaged with confocal, others with light-sheet; some use nuclear stains, others membrane markers. Such heterogeneity makes cross-tissue comparison unreliable.
3. **Inconsistent Z-stack coverage**
Different thicknesses give different apparent density of “modules,” confounding segmentation.

Extended Data Figure 2 — Segmentation Validation

Critique

1. **Lack of ground truth**
Validation relies on algorithm-algorithm agreement rather than algorithm–expert annotations.
2. **Over-reliance on IOU (intersection over union)**
IOU alone cannot assess biological correctness; many false-positive segmentations would still yield high IOU if boundaries are uniformly shifted.
3. **Missing inter-sample variability**
Only two tissue replicates per condition are shown, far below what is necessary.

Extended Data Figure 3 — Clustering Parameter Sweep

Critique

1. **Parameter ranges too narrow**
The authors sweep hyperparameters only in small windows, effectively guaranteeing that clusters remain stable. No stress testing is performed.
2. **No sensitivity analysis**
Without sensitivity curves, we cannot evaluate whether cluster boundaries are algorithmically stable.
3. **Possible manual selection**
The chosen parameter ranges appear tuned to reach visually appealing clusters (modules), creating confirmation bias.

Extended Data Figure 4 — Developmental Comparisons Using Additional Markers

Critique

1. **Markers not orthogonal**
Many markers label overlapping cell populations, making it unclear whether changes reflect developmental progression or marker reactivity.

2. **Lack of quantitative developmental trajectories**

No dimensionality reduction or pseudo-time analysis is provided; developmental claims remain speculative.

Extended Data Figure 5 — Mechanical Simulations (Supplementary Models)

Critique

1. **Parameter non-identifiability**

Many simulations produce similar output patterns. Without identifying the solution space, authors cannot argue that real tissues uniquely match simulated modules.

2. **Simplified boundary conditions**

Simulations assume uniform tissue elasticity—biologically unrealistic.

3. **Scale mismatch**

Simulation mesh resolution is orders of magnitude lower than cellular dimensions, inducing aliasing artifacts.

Extended Data Figure 6 — Perturbation Controls

Critique

1. **Controls insufficient**

Only short-duration controls are shown. Some perturbations require long-term controls, especially ECM modifications.

2. **Cell viability not monitored**

Effects attributed to “module disruption” may simply reflect widespread apoptosis.

Extended Data Figure 7 — Tissue-Specific Examples of Modules in Understudied Organs

Critique

1. **Highly inconsistent staining quality**

Some tissues show weak staining, making boundaries appear artificially smooth.

2. **Low resolution in several images**

Low-quality images inflate the appearance of “modules,” especially where resolution is insufficient to resolve cellular detail.

Extended Data Figure 8 — Comparative Anatomy Across Species

Critique

1. **Species chosen opportunistically**
The authors choose species whose tissues are already known to exhibit strong modular patterns.
2. **Missing phylogenetic correction**
Without phylogenetic adjustment, cross-species comparisons are not meaningful.

Extended Data Figure 9 — Additional Functional Assays

Critique

1. **Functional assays too coarse**
Bulk ATP flux and contractility do not demonstrate module-specific function.
2. **No spatial activity mapping**
Calcium imaging or optogenetics would be necessary to show functional modularity.

Extended Data Figure 10 — Alternative Segmentation Methods

Critique

1. **Alternative methods appear poorly optimized**
This artificially improves the relative performance of the authors' preferred segmentation method.
2. **Training datasets not shared**
Without training data, reproducibility is compromised.

Extended Data Figure 11 — Statistical Analyses of Module Size Distributions

Critique

1. **Improper statistical tests**
The authors apply Gaussian fits to clearly non-Gaussian distributions.
2. **Missing multiscale analysis**
If mesoscale modules exist, they should manifest across scales; such analysis is absent.

Extended Data Figure 12 — Module Dynamics Under Stress

Critique

1. **Stress conditions are extreme**
Using excessively high shear or chemical stress induces artifacts unrelated to physiological function.
2. **Imaging fails to control for phototoxicity**
Phototoxicity itself may alter module structure.

Extended Data Figure 13 — 3D Reconstructions of Tissue Blocks

Critique

1. **Voxel anisotropy ignored**
Z-resolution is much poorer than XY resolution, making 3D module shapes unreliable.
2. **Surface smoothing artifacts**
Reconstruction pipelines over-smooth boundaries, artificially regularizing module geometries.

Extended Data Figure 14 — Cross-Organ Machine Learning Classification

Critique

1. **Possible overfitting**
No test on unseen organs; classification performance may simply memorize features.
2. **Feature attribution missing**
Without SHAP or saliency maps, it is unclear what features drive classification.

Extended Data Figure 15 — Extended Integrative Model

Critique

1. **Speculative diagram**
Adds layers of complexity without supporting data.
 2. **No predictive power**
Does not generate testable hypotheses.
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4. Supplementary Figures: Critical Review

Chen *et al.* rely heavily on Supplementary Figures to justify decisions in segmentation, clustering, dimensionality reduction, and perturbational interpretation. Below are key issues.

Supplementary Figure 1 — Raw Data Preprocessing

Critique

- “Batch correction” applied to imaging data is not described mathematically.
- Aggressive filtering may remove authentic biological heterogeneity.

Supplementary Figure 2 — Algorithm Stability Tests

Critique

- Stability is evaluated only on synthetic data.
- Real tissue data test is missing, rendering these results irrelevant.

Supplementary Figure 3 — Marker-Specific Module Validation

Critique

- Markers are not validated for specificity.
- Some markers are pan-tissue, making it impossible to distinguish cell-type-specific modules.

Supplementary Figure 4 — Calcium/Functional Data

Critique

- Imaging fields are small and may not capture entire modules.
- No replication (n stated but raw data missing).

Supplementary Figure 5 — Computational Model Architecture

Critique

- Model inputs are ill-defined; authors do not provide graph topology or adjacency matrix rules.
- No code available; therefore, no reproducibility.

Supplementary Figure 6 — Expanded Species Comparisons

Critique

- Many species have very different tissue architectures; mapping them to the same “module” definition oversimplifies biology.

Supplementary Figure 7 — 4D Timelapse Reconstruction

Critique

- Motion correction artifacts create pseudo-periodic patterns easily mistaken for modules.

Supplementary Figure 8 — Additional Perturbation Assays

Critique

- No dose-response curves.
- No control for nonspecific toxicity.

Supplementary Figure 9 — Cross-Modality Validation

Critique

- Authors correlate transcriptomics with imaging, but spatial transcriptomics resolution is insufficient to resolve “modules.”

Supplementary Figure 10 — Expanded Functional Modeling

Critique

- Claims that modules serve as “gates” or “valves” are overstated and not supported by quantitative data.

5. Integrated Critique and Conceptual Discussion

5.1. Lack of a Reproducible, Quantitative Definition of Modules

The central flaw of the study is the absence of a rigorous, falsifiable definition of a “mesoscale module.” Without specifying:

- geometric constraints
- cell-type composition limits
- dynamical stability

- functional necessity

the term becomes **malleable** and **descriptive rather than mechanistic**.

5.2. Heavy Reliance on Imaging Artifacts

Across figures, module boundaries resemble:

- segmentation biases
- smoothing artifacts
- thresholding steps
- resolution limitations
- pseudo-periodic patterns created by optics

Without raw data and independent validation, these concerns remain unresolved.

5.3. Insufficient Perturbational Evidence

The claim that modules are *functionally indispensable* is not supported because:

- perturbations are nonspecific
- functional assays are bulk
- module disruption is not isolated from global tissue injury.

5.4. Overgeneralization Across Tissues and Species

The authors extrapolate limited observations to propose that **all animal tissues** operate via mesoscale modules. Yet tissues such as:

- liver
- spleen
- bone marrow
- endocrine organs

have diffuse architectures inconsistent with the presented model.

5.5. Statistical Underpowering

Across main, ED, and supplementary figures:

- sample sizes are not reported

- batch effects ignored
- multiple testing not corrected
- effect sizes missing
- confidence intervals absent
- many plots lack replicates

This undermines the quantitative conclusions.

6. Conclusion

Chen *et al.* present an ambitious framework proposing that “mesoscale modules” represent fundamental organizational units bridging single cells to organs. However, the evidence remains **largely correlative, underspecified, and over-interpreted**. The absence of rigorous definitions, missing perturbation-causal demonstrations, lack of reproducible computational methodology, and substantial imaging/segmentation artifacts significantly weaken the central claims. More careful experimental design, quantitative validation, functional testing, and theoretical clarity are necessary before “mesoscale modules” can be accepted as a fundamental biological principle.

Reference

- 1 Chen, Y. *et al.* Bridging single cells to organs: Mesoscale modules as fundamental units of tissue function. *Cell* **188**, 6393-6410 (2025).
<https://doi.org/10.1016/j.cell.2025.10.012>