differences should also become possible. Although some phenotypic differences reflect cell-autonomous variability, a substantial fraction is likely emergent from the relationships between cells. Uncovering the logic of how these cell-cell relationships contribute to tissue function is an important avenue opened up by these integrative methods and the data underlying them.

An important area for technical improvement in analysis methods rests on the fact that current assessments are quite qualitative in nature. Although this does not place a direct limit on the efficacy of methods, it does place a limit on our understanding of how best to apply them or improve upon them. Spatial clustering methods or identification of spatial distributions of cell types, for example, are often visualized with microscopy images and are said to be good representations when these computationally defined features match the cytoarchitecture and morphology of the tissue. There are some popular statistical measures, such as those for determining spatial autocorrelation, but these do not capture the performance of all classes of spatial analysis tasks. In addition to the advances in spatial analysis represented by Tangram and SpaGCN, other spatial tools, not detailed here, are also useful. As with any new field, to better understand the pros and cons of the many spatial analysis tools, an independent, rigorous and quantitative benchmarking across spatially resolved transcriptomics analysis tools is needed.

Moving forward, tools such as SpaGCN[2] and Tangram[1] will be invaluable in establishing spatial regions directly derived from gene expression data, rather than defined from traditionally agreed anatomical boundaries. Although gene expression need not be the be-all and end-all, it provides a unified and quantitative framework to link activity at the cellular and tissue levels. Boundaries defined from spatial expression will link processes such as cell-cell communication, cell migration and morphogenesis in organ formation. Analysis tools for spatially resolved transcriptomics usually take a data-first approach to understanding biology, sometimes described as 'unbiased', but integration with existing biological knowledge to understand causal mechanisms will ultimately require testable hypotheses in combination with high-quality data.

Particularly important for future study are questions relating to evolution and development, as well as their interplay, as modular expansion of spatial domains to create new functions is a repeated theme of both. Evolution and development offer a vast space from which to collect data, with a new class of integration to consider, for which systematic tools such as SpaGCN and Tangram will be essential. Although these tools can capture biological phenomena such as morphological patterns in the brain, clusterings have difficulty in distinguishing between byproducts of evolution and phenotypic traits that are the direct products of selection. Spatial expression across development should provide valuable insight into molecular mechanisms, whereas spatial expression across species helps to capture selection and conservation.

The rapid parallel development of molecular tools available both in spatial genomics[5] and in lineage tracing and clonal identification[10] will, together with computational methods like SpaGCN and Tangram, enable a new era of experimental design and discovery. Spatially resolved transcriptomics has the potential to be the revolution of this decade, much as single-cell techniques were for the previous one; these analysis tools will help to realize that potential. ❐

Shaina Lu, Daniel Fürth and Jesse Gillis ✉

Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, USA.

✉*e-mail: JGillis@cshl.edu*

Published online: 28 October 2021
https://doi.org/10.1038/s41592-021-01272-7

References

1. Scalia, G. et al. *Nat. Methods* https://doi.org/10.1038/s41592-021-01264-7 (2021).
2. Hu, J. et al. *Nat. Methods* https://doi.org/10.1038/s41592-021-01255-8 (2021).
3. Svensson, V., Teichmann, S. A. & Stegle, O. *Nat. Methods* **15**, 343–346 (2018).
4. Edsgärd, D., Johnsson, P. & Sandberg, R. *Nat. Methods* **15**, 339–342 (2018).
5. Asp, M., Bergenstråhle, J. & Lundeberg, J. *Mol. Cell. Dev. Biol.* **42**, e1900221 (2020).
6. La Manno, G. et al. *Nature* **596**, 92–96 (2021).
7. Di Bella, D. J. et al. *Nature* **595**, 554–559 (2021).
8. Srivatsan, S. R. et al. *Science* **373**, 111–117 (2021).
9. Regev, A. et al. *eLife* **6**, e27041 (2017).
10. Wagner, D. E. & Klein, A. M. *Nat. Rev. Genet.* **21**, 410–427 (2020).

Acknowledgements

S.L. is supported by the Edward and Martha Gerry Fellowship funded by The William Stamps Farish Fund and the Gladys and Roland Harriman Foundation. D.F. is supported by a NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation. J. G. is supported by NIH grants R01MH113005 and R01LM012736.

Competing Interests

The authors declare no competing interests.

ORGANOIDS

Towards spheroid-omics

The MISpheroID knowledgebase records and organizes experimental parameters from thousands of cancer spheroid experiments, revealing heterogeneity and a lack of transparency in key spheroid research reporting practices.

Timothy L. Downing

For more than 40 years, researchers have explored the development of cell culture models that recapitulate biological processes as they occur within three-dimensional (3D) physiological contexts. However, within the past 10 years, there has been a sharp increase in the rate of spheroid studies published, owing to the valuable insights that these models provide into cancer pathophysiology (including cell migration and matrix invasion), as well as pharmacological response through drug testing[1]. 3D spheroid cultures are established through the aggregation of suspended (non-adherent) cells derived

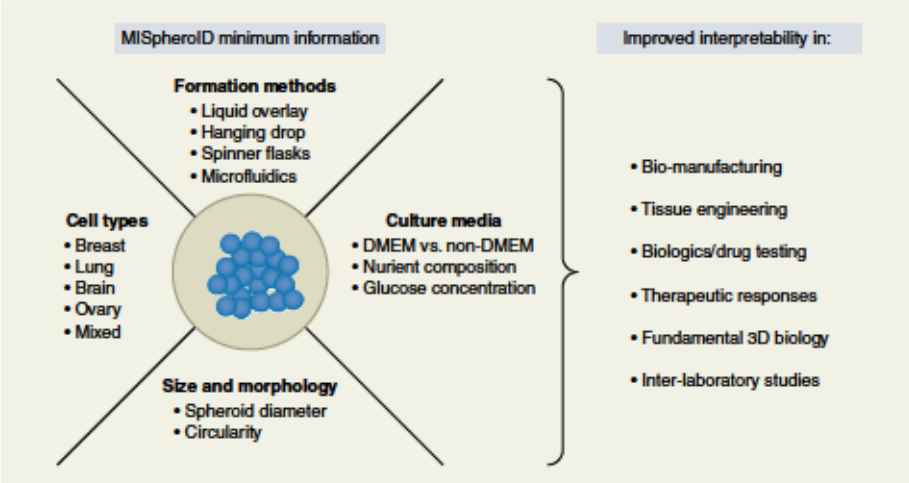

Fig. 1 | Recommended minimum information for spheroid culture. An analysis of published literature and empirical studies revealed four components of spheroid culture assembly (cell type, culture media, formation method and size) that vary widely between experiments and impact spheroid research outcomes. Through the MISpheroID tool, researchers can upload data from their spheroid experiments along with a description of the four components as a set of recommended minimum information. This standardization in reporting practices is anticipated to improve the interpretability of study results, thereby benefiting many applications of spheroid research.

from tumors. These 3D cultures have been particularly useful in recreating aspects of tumor physiology, including the emergence of biomolecular gradients in oxygen and nutrient availability, acidity and metabolic waste products, as well as heterogeneity in the extracellular matrix cues and mechanical forces experienced by cells (through cell–cell and cell–matrix interactions) within solid tumors, all of which are known to have profound impacts on tumor biology[2]. In this issue of *Nature Methods*, Peirsman and Blondeel et al.[3] reveal diversity in spheroid generation protocols and highlight a lack of transparency in reporting standards.

As the number of spheroid experiments performed continues to grow, the spheroid research community is poised to accelerate discovery through community-wide data consolidation and knowledge sharing. However, such efforts require widespread adoption of research standards in reporting (in both experimental methods and outcomes) to ensure inter-laboratory interpretability. While there have been several efforts to establish a set of 'minimum information' for biology and biomedical experimental investigation (for example, the Minimum Information for Biological and Biomedical Investigations (MIBBI))[4], there is no consensus or evaluation of minimum information criteria appropriate for spheroid research.

Here, Peirsman and Blondeel et al. assessed research reporting practices in spheroid research through a bibliographic screen of studies published within the National Library of Medicine's public database, using the MEDLINE (PubMed) search interface. Study conditions were coded and formulated into a digitally curated database of spheroid experiments with annotations across 98 parameters related to spheroid setup, characterization and application, collectively giving rise to the MISpheroID knowledgebase (https://www.mispheroid.org). Using this knowledgebase, the authors performed an initial analysis of reporting practices within breast cancer spheroid-related studies that revealed widespread heterogeneity and/or a lack of transparency in reporting across three critical spheroid culture parameters: culture medium, spheroid formation method and spheroid size. For example, nearly half of the breast cancer spheroid experiments captured in the literature screen neglected to report culture medium glucose concentration, and while microscopy tools were utilized in 87.8% of experiments, a much smaller percentage of studies (23.3%) reported information on spheroid size and/or morphology metrics, both of which have been shown to affect spheroid study conclusions[5,6]. Notably, these deficiencies in reporting were also observed across experiments using spheroids derived from other tumor tissues (such as brain, liver, colon, lung, ovary and pancreas). These observations — along with evidence of a growing collection of complex spheroid formation methods[2,7], including liquid overlay, hanging drop, spinner flasks, magnetic levitation or microfluidic apparatus — further highlight the need for established minimum information reporting standards that are tailored specifically to spheroid research requirements.

Peirsman and Blondeel et al. propose four components of spheroid assembly and culture as minimum information: (1) cell type; (2) culture medium; (3) size; and (4) formation methods. These contribute to tumor-specific biology, nutrient and energy source availability, diffusion of biomolecules, and spheroid assembly and structure, respectively, and show clear deficiencies and/or heterogeneity in reporting. To examine the legitimacy of these components as appropriate recommendations for minimum information reporting standards, the authors empirically evaluated the impact of the components on characteristics of spheroid culture through transcriptome-wide expression profiling (RNA-seq).

Principal component analysis of gene expression revealed that two of the five cancer cell lines examined (one derived from the lung and the other from the ovary) showed widespread changes in gene expression patterns in response to culture medium type. The A549 lung cancer cell line showed strong expression sensitivity to basal medium conditions (Dulbecco's modified Eagle's medium (DMEM) versus non-DMEM), while the divergence in global expression patterns in the ovarian cancer cell line (SKOV3) due to medium conditions was less clear. Conversely, one glioblastoma cell line (U87MG) showed relatively little divergence in expression across the medium conditions tested. Gene set enrichment analysis revealed that genes dynamically regulated in response to culture medium variations were enriched for several molecular signatures defined by the MsigDB molecular signature database, including those involved in angiogenesis, DNA repair, interferon response, metabolism, protein secretion, hypoxia response, proliferation and other tumor-associated signaling (that is, PI3K–AKT–mTOR, TGF-β and K-RAS).

Further examination of medium-dependent effects on spheroid properties (beyond gene expression) revealed several notable changes to characteristics known to impact spheroid (or tumor) physiology. Nutrient-poor media conditions (for example, Eagle's minimum essential medium (EMEM)) drove increases in cell death in some cell lines, which was accompanied by reduced metabolic activity as measured by ATP content. Glucose concentration

impacted lactate secretion to glucose uptake (L/G) ratios and led to changes in inflammatory and angiogenic protein secretion. The level of change observed in ATP content in response to cancer 'treatment' (for example, radiotherapy) was also heavily impacted by the culture medium used to establish spheroids. Notably, spheroid size correlated significantly with ATP content and necrotic zone formation. While these trends were observed across spheroids derived from multiple cell lines, most effects were cell-type-dependent and, in some cases, showed inverse trends across identical media conditions.

Finally, to confirm the utility of the four components recommended as minimum information for spheroid culture assembly by the MISpheroID consortium, the authors performed spheroid experiments across seven independent laboratory sites; all seven sites used a common HCT116 colon cancer cell line (although at variable passage numbers) and examined the impact of six different culture medium conditions (while keeping other factors constant). Although there was high correlation in data patterns across all sites (Spearman correlation >0.9), there was, perhaps unexpectedly, some site-to-site heterogeneity in certain spheroid characteristics. Notably, despite this heterogeneity, the authors were able to ascertain generalizable trends regarding the relative impact that different medium conditions had on spheroid circularity, size and cell death. Together, these studies demonstrate the potential utility of community-wide adoption of the minimum information reporting standards established through the MISpheroID consortium. The consortium defines a four-component spheroid string ID (MISpheroID string) (for example: [cell type–culture medium–formation method–size]) through which spheroid experiments can be cataloged accordingly for effective inter-laboratory interpretation, comparison and replication.

As a powerful tool for the evaluation of experimental consistency with a straightforward framework for exchanging resources and understanding data output, the MISpheroID online portal establishes a thorough knowledgebase of more than 3,000 spheroid experiments that will grow through continued engagement amongst researchers, reviewers and journal editors. The authors of this study envision those users interacting directly with the MISpheroID tool to ensure published experimental data contain minimum information appropriate for spheroid research, making possible omics-style (systematic, comprehensive and parameterized) investigations of spheroid biology that could usher in a more compendious understanding of how to interpret and build on the collective knowledgebase from experiments performed throughout the spheroid research community (Fig. 1). ❐

Timothy L. Downing [1,2,3,4] ✉

[1]Department of Biomedical Engineering, University of California Irvine, Irvine, CA, USA. [2]Department of Microbiology and Molecular Genetics, University of California Irvine, Irvine, CA, USA. [3]UCI Edwards Lifesciences Foundation Cardiovascular Innovation and Research Center (CIRC), University of California Irvine, Irvine, CA, USA. [4]NSF-Simons Center for Multiscale Cell Fate Research, University of California Irvine, Irvine, CA, USA.

✉*e-mail: tim.downing@uci.edu*

Published online: 3 November 2021
https://doi.org/10.1038/s41592-021-01311-3

References

1. Vinci, M. et al. *BMC Biol.* **10**, 29 (2012).
2. Kamatar, A., Gunay, G. & Acar, H. *Polymers* **12**, (2020).
3. Petrsmart, A. et al. *Nat. Methods* https://doi.org/10.1038/s41592-021-01291-4 (2021).
4. Taylor, C. F. et al. *Nat. Biotechnol.* **26**, 889–896 (2008).
5. Zanoni, M. et al. *Sci. Rep.* **6**, 19103 (2016).
6. Vande Voorde, J. et al. *Sci. Adv.* **5**, eaau7314 (2019).
7. Vadivelu, R. K. et al. *Micromachines* **8**, 94 (2017).

Acknowledgements

This work was supported by grants from the National Institutes of Health (NIH) National Cancer Institute (NCI) (New Innovator Award DP2CA250382-01), the National Science Foundation (NSF) (DMS1763272) and the Simons Foundation (594598 QN).

Competing Interests

The author declares no competing interests.