Illuminating CRISPR Screening in Intestinal Organoids with Live-Cell Imaging

An Interview with Thomas Dennison, Research Associate, Milner Therapeutics Institute & Zilbauer Lab, Cambridge Stem Cell Institute

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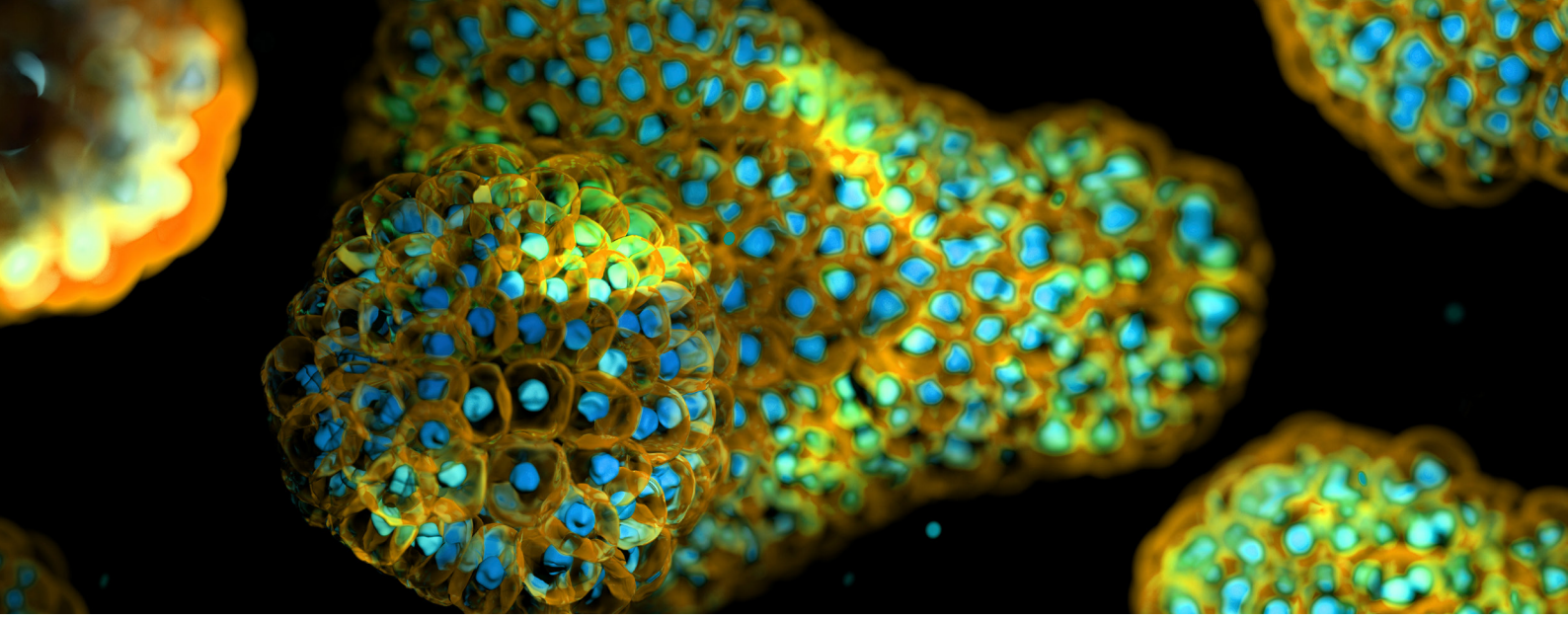
Live-cell imaging is increasingly used to support CRISPR screening in organoid systems, supporting more relevant phenotypes for disease modeling and target validation. To understand how this works in practice, we spoke with Thomas Dennison, a Postdoctoral Research Associate at the Milner Therapeutics Institute, about how the Incucyte® Live-Cell Analysis System and its Organoid Software Module fit into his workflow from experimental setup through analysis.



Q: How are intestinal organoids incorporated into your CRISPR screening workflow? What factors led you to use organoids rather than 2D models?

Thomas: Our lab is interested in the role of intestinal epithelium in Inflammatory Bowel Disease (IBD). IBD is a highly complex and heterogeneous disease- no two patients are alike. Traditional models, such as mice or 2D epithelial cell lines, reflect only narrow aspects of the condition and fail to capture its complexity and diversity.

For this reason, we have adopted patient-derived intestinal epithelial organoids as our model of choice. We grow these cultures directly from either foetal or paediatric patient biopsies into 3D structures that reflect in vivo architecture and dynamics. Critically, they retain the donor's genetic and epigenetic background, allowing us to model not only healthy versus diseased tissue, but the full spectrum of patient variation.

Over recent years, we have generated and profiled organoids from a large cohort of IBD patients and healthy controls to identify distinct molecular signatures linked to disease subtypes. However, the functional tools for testing these signatures lag behind what's possible in simpler systems. Our work aims to close that gap by developing scalable, arrayed CRISPR screening workflows that let us systematically test the functions of the genes and pathways highlighted in those profiling studies.

Q: What kinds of CRISPR targets or pathways are you exploring in intestinal organoids? What are you most excited about from the live-cell imaging studies so far?

Thomas: Our recent paper in Gut identified a subset of Crohn's disease patients with reduced DNA methylation across genes within the MHC class I pathway. Particularly intriguing was the fact that this pattern seems to be linked with long-term patient outcome. We are now adapting CRISPR screening methodologies to dissect how this pathway is regulated in the intestinal epithelium in health and how it may be disrupted in disease. Ultimately, we hope this work will identify novel therapeutic targets and uncover prognostic biomarkers.

Q: How does continuous live-cell imaging fit into your assays day to day? Has it changed how you capture or interpret phenotypes over time?

Thomas: A major strength of live-cell imaging is its ability to integrate seamlessly with our existing assays. We can now continuously monitor organoid growth dynamics as part of existing workflows, rather than relying on complicated time-course experiments that have to be performed separately. This adds an important layer of quantitative data with minimal extra effort.

This extra data layer has been invaluable to our efforts to optimise gene-editing protocols. Continuous growth monitoring has allowed us to identify conditions that balance optimal efficiency with minimal impact on cell health. For screening itself, multiplexing live brightfield with end-point high-content imaging assays adds depth to our phenotyping. The prospect of more advanced image analysis techniques

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opens the door to take this even further, potentially allowing us to assess the impact of perturbation on real-time tissue dynamics.

Q: The Incucyte® Organoid Software Module tailors analysis specifically for organoid morphology and growth kinetics. How has this tool impacted your data accuracy and throughput in CRISPR screening?

Thomas: Throughput has always been a major bottleneck in organoid research, due to their 3D nature. The Organoid Software Module is a significant step towards changing that. It allows us to generate plate-based, high-throughput data from brightfield images whilst preserving the 3D structure that makes organoids such valuable tools. The quantitative use of brightfield data means it can be easily integrated with other assays, so we can collect kinetic and end-point data in parallel. Furthermore, it adds a level of objectivity and reproducibility to image analysis which can otherwise be lacking. The ongoing adaptation of this software to novel culture platforms, such as the 3D nanowell plate function, further adds to the scalability of organoid research.

Q: In what ways is the combination of CRISPR screening and live-cell imaging enhancing your understanding of intestinal stem cell biology, particularly in the context of disease modelling or therapeutic development?

Thomas: We are really excited about the prospects for our approach. Bringing together the patient relevance of the organoid model, the functional insights of CRISPR screening and the throughput of cutting-edge imaging technologies is opening avenues that were previously not possible in IBD research.

In our immediate context, we can now start to functionally interrogate the epigenetic mechanisms of the epithelium in a model that reliably reflects the situation in vivo. From a disease perspective, this has the potential to shed light on the long-term dynamics of IBD on a patient-to-patient level. Our partnership with the MRC-Milner-AstraZeneca functional genomics screening laboratory is also beginning to identify novel drug targets for future development, as well as a possible basis for a prognostic test. These approaches could also be adapted for other diseases and organoid models as well.

Q: Looking ahead, how do you see live-cell imaging evolving to further support complex genome editing workflows in organoids? Are there capabilities you'd like to see?

Thomas: It is an exciting time to be involved in organoid research, with new technologies emerging all the time. The new CX3 platform is a case in point. Automating real-time fluorescence readouts in organoids has been a challenge in the field due to challenges with their 3D structure. The CX3 is a big step forward here and has the potential to greatly enrich CRISPR screening readouts.

The integration of machine-learning and AI-based image analysis into organoid research could really allow us to more fully exploit the complexity of organoid models. The ability to quantify subtle changes in the 3D structure and dynamics of

the crypt-villus axis would add data layers not previously accessible.

Finally, I believe the next frontier in 'omics research will be cohort-scale functional genomics- generating functional data across populations of patient-derived organoids rather than single models. Technological solutions will be central to achieving this, requiring continued interplay between imaging platforms and culture approaches to achieve scale. This level of data could have profound implications for complex diseases such as IBD, providing an opportunity to uncover the diverse network of diseases mechanisms that so often remain obscure with traditional approaches.

Learn more about the Incucyte® CX3 Instrument

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