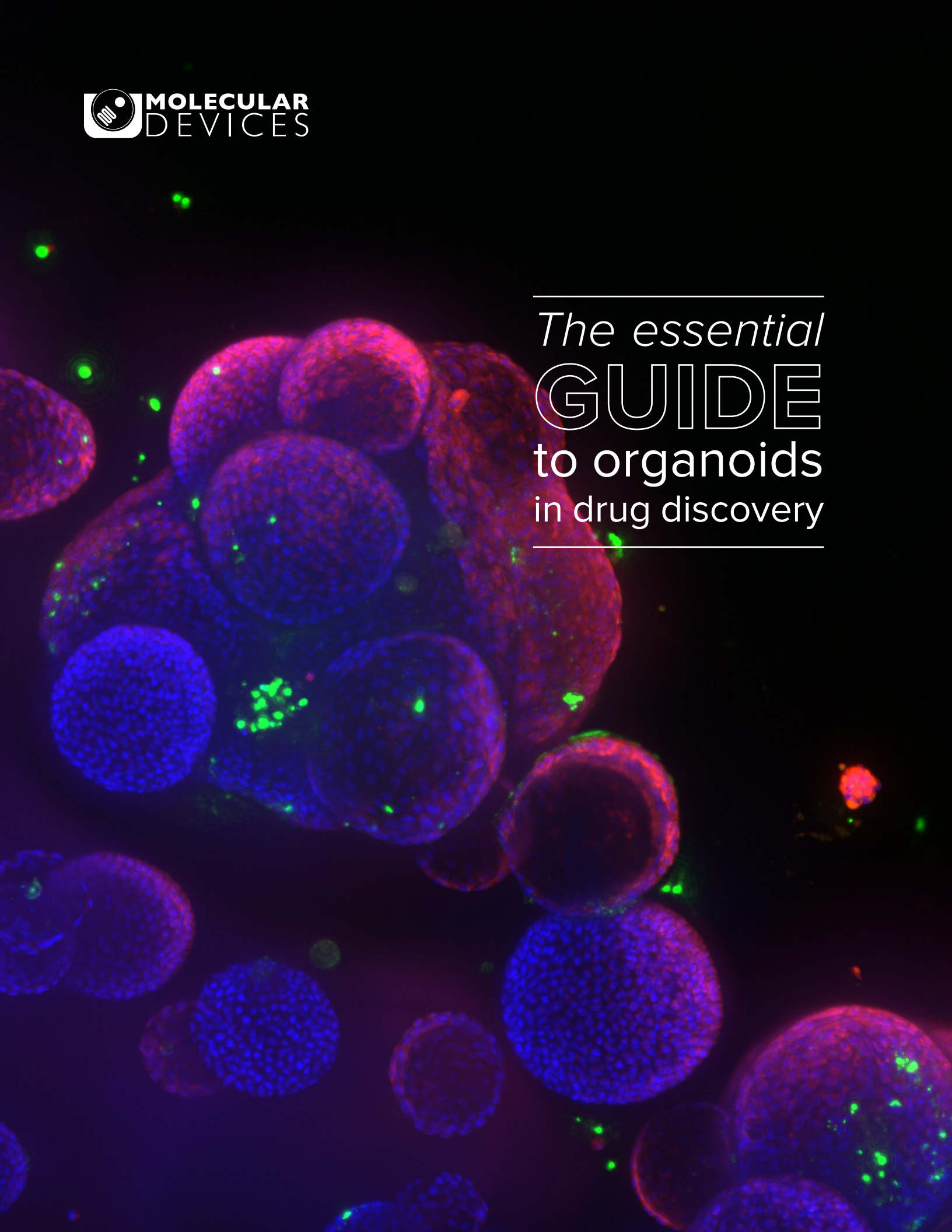
MOLECULAR DEVICES



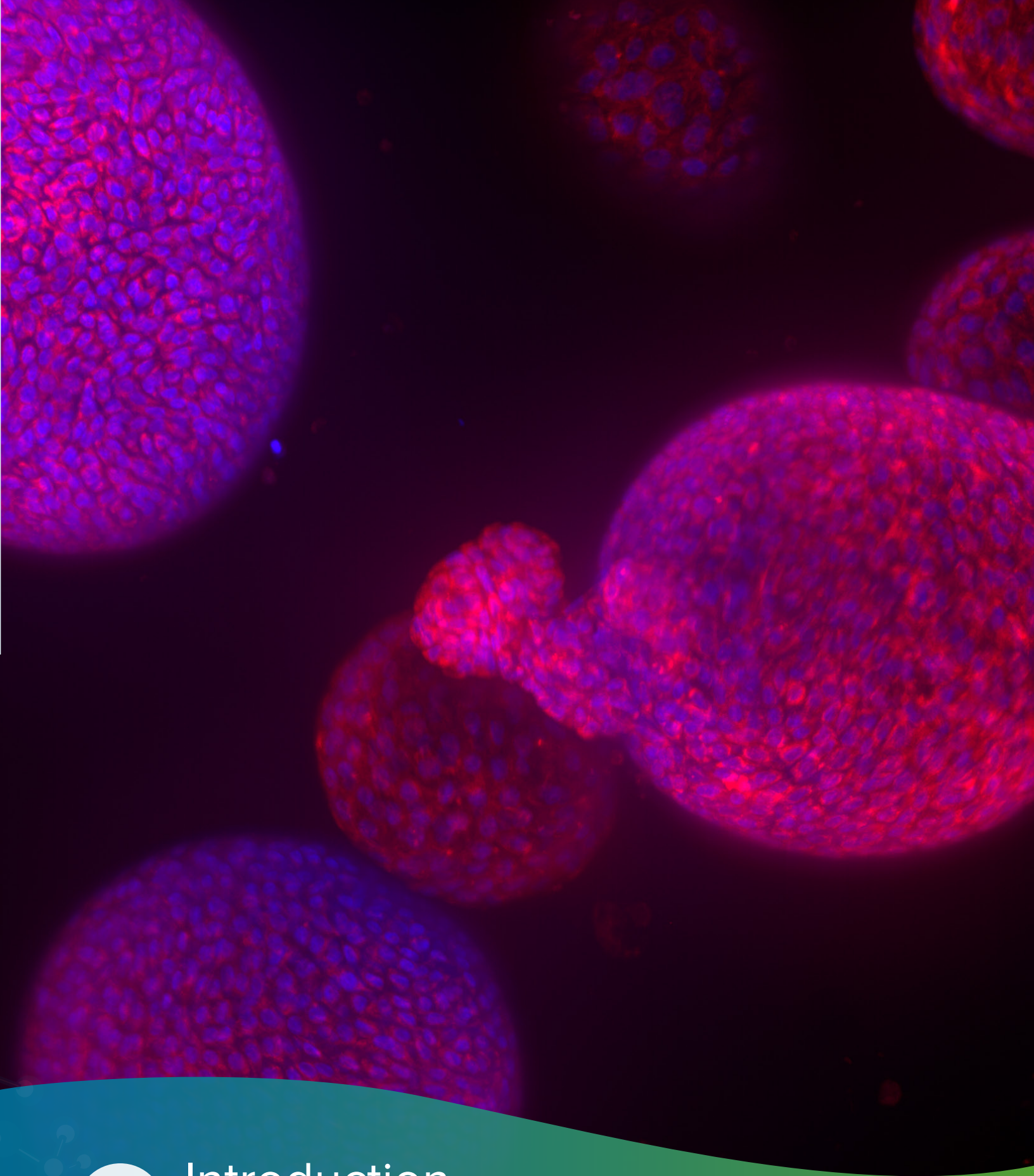
The essential GUIDE to organoids in drug discovery

Overview

Three-dimensional (3D) organoid development is one of the most important advancements in drug discovery research to date. The ability to self-organize and mimic functional organ cell types is believed to better represent in vivo biology than 2D monolayer cell cultures. As such, organoids are becoming increasingly important in the fields of cancer research, neurobiology, stem cell research, and drug discovery—providing important insights into the mechanisms of tissue development and disease and present a very valuable approach for biological research and drug development.

Fueled by rapid developments in advanced cell technologies and the desire to create models that closely resemble the in vivo tissue, organoids have undeniably taken their rightful place at the forefront of disease modeling and drug discovery. Developed to represent many different organs of the human body, with applications expected to grow over the next few years, organoids form the foundation of innovations yet to come in precision, translational, and regenerative medicine.

Contents



1 Introduction to organoids

Introduction to organoids

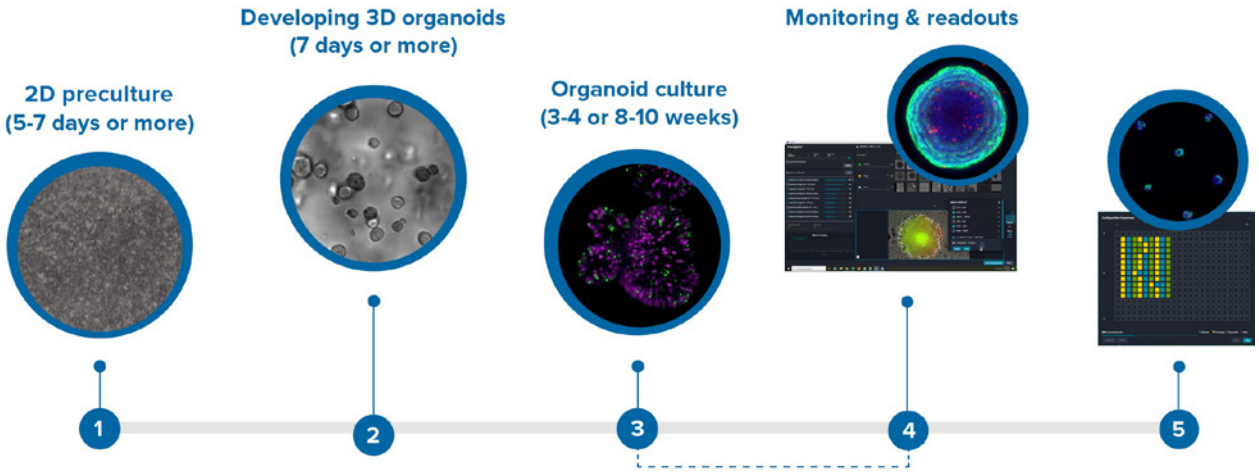
a. What defines an organoid?

Interestingly, the idea of organoids is not new. Today's organoid technology is the product of decades of research. In fact, the foundations of the concept go back to the beginning of the 20th century. In general, the word organoid was used as an extension of 3D cultures, referring to tissue fragments from organs that were grown in 3D gels.[1] Here, we refer to organoids as multi-cellular microtissues derived from stem cells or organ progenitors that self-organize and closely mimic the complex structure and function of human organs.[2]

Learn more about **the history of organoids** here.

b. General workflow for organoid research

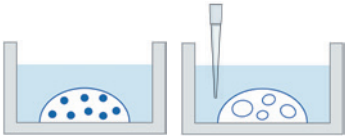
Culturing organoids is more challenging compared to conventional 2D monolayer cell cultures. There are some similarities between 2D and 3D culture, however, additional considerations are required, such as the availability of tissue-specific medium, the use of components subject to batch-to-batch variations (such as Matrigel) and the extended time required to culture mature organoids. The process typically starts with a 2D preculture for cell expansion, then cells are seeded in an extracellular matrix (Matrigel or hydrogel) and grown in a suitable media to start the 3D culture. Throughout the process, differentiation of the organoids is regulated by the mixture of growth factors and triggering signaling pathways to guide cellular differentiation to their specific cell fate. Due to the complexity of organoids, more sophisticated 3D imaging and analysis techniques are needed to characterize these biological assays accurately and efficiently. Today, automated confocal imaging systems and 3D image analysis software are commonly used to help researchers streamline their workflow and obtain optimal results.



2D expansion

Typically, iPSC-derived or primary tissue cells precultured or expanded in 2D culture.

Matrigel dome 3D



Cells mixed with ECM/VME (such as Matrigel), and droplets placed into plates, form a solid droplet "dome."

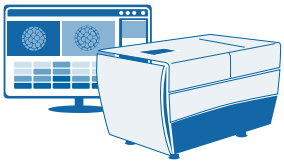
Media is added to promote cell growth and differentiation.

Some organoids are grown without ECM.

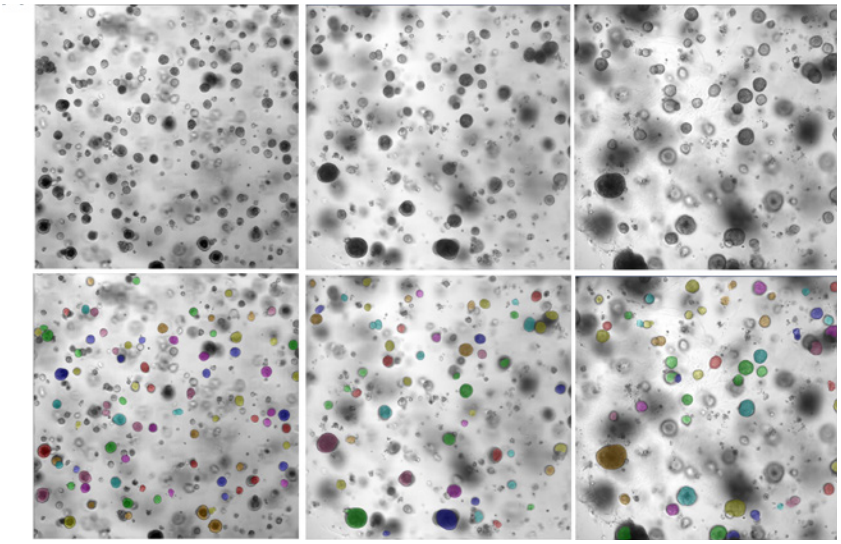
Monitoring organoid growth & development

Development and growth of organoids can be monitored by automated imaging that allows evaluation of organoids size, volume, and complexity.

Confocal imaging & 3D analysis

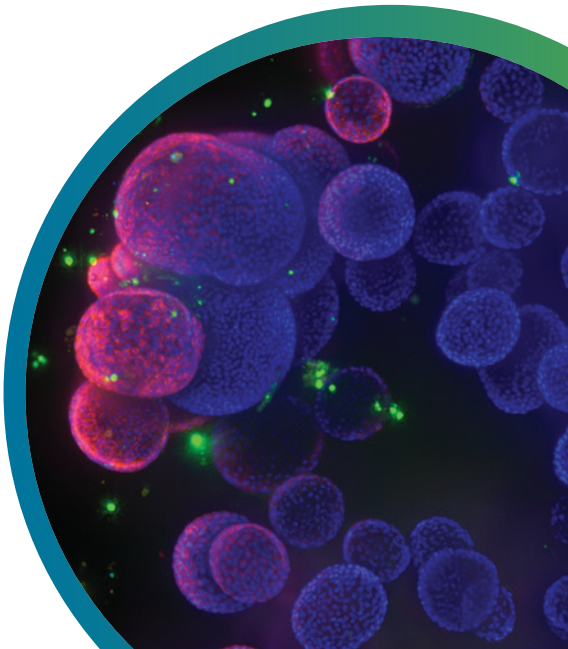


Confocal imaging and 3D analysis allows for quantitative characterization of cellular content as well as count and measurement of cells.



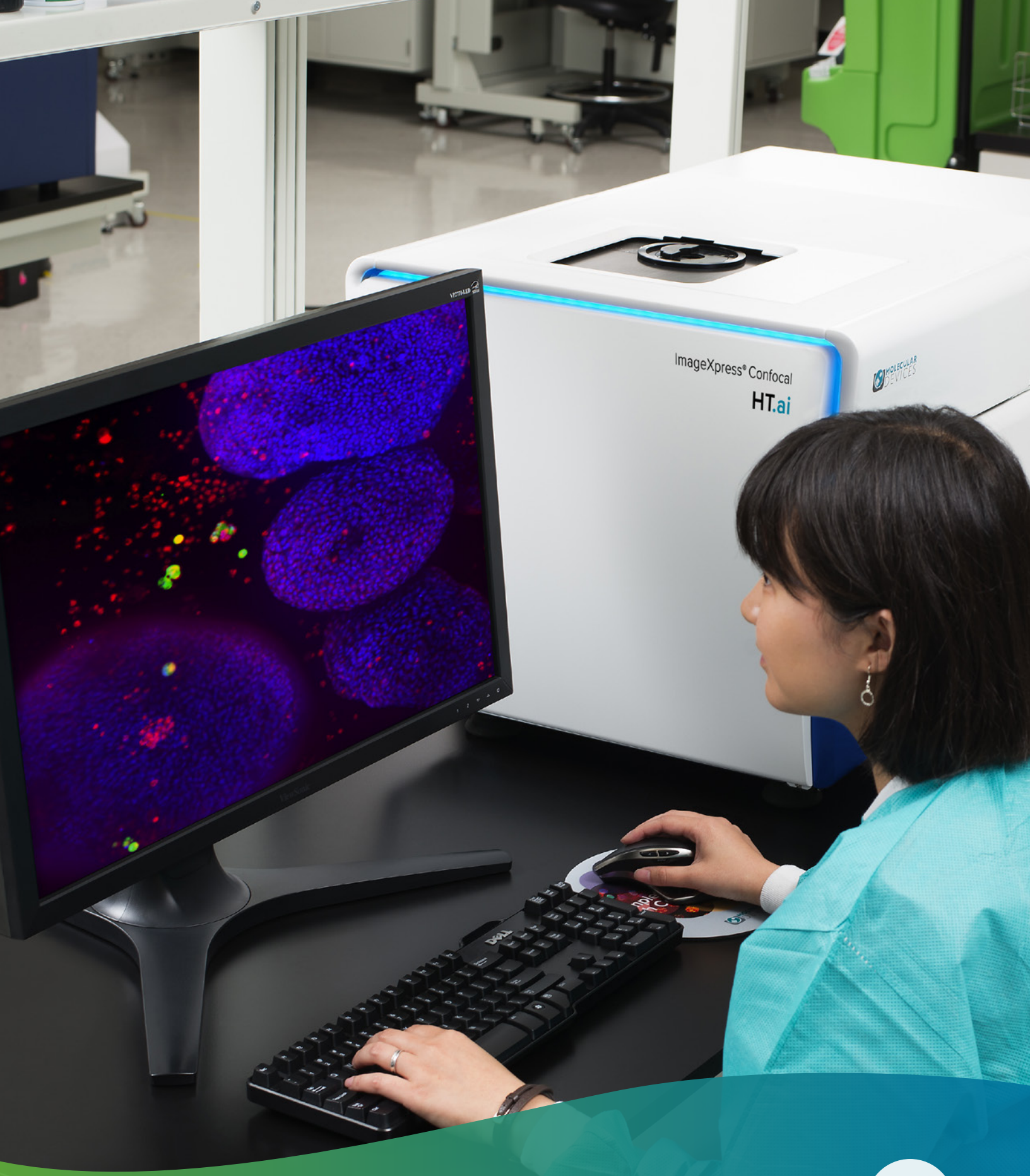
Week 2 Week 3 Week 4

Applying AI-based method to analyze lung organoids. Images of lung organoids grown in Matrigel dome. These images usually have high, non-homogenous background which prevents robust object segmentation. Using a deep learning-based segmentation tool in IN Carta (SINAP), a model was created to segment lung organoids (mask shown in colored overlay). This approach allows for label-free image analysis for monitoring growth of organoids in culture.

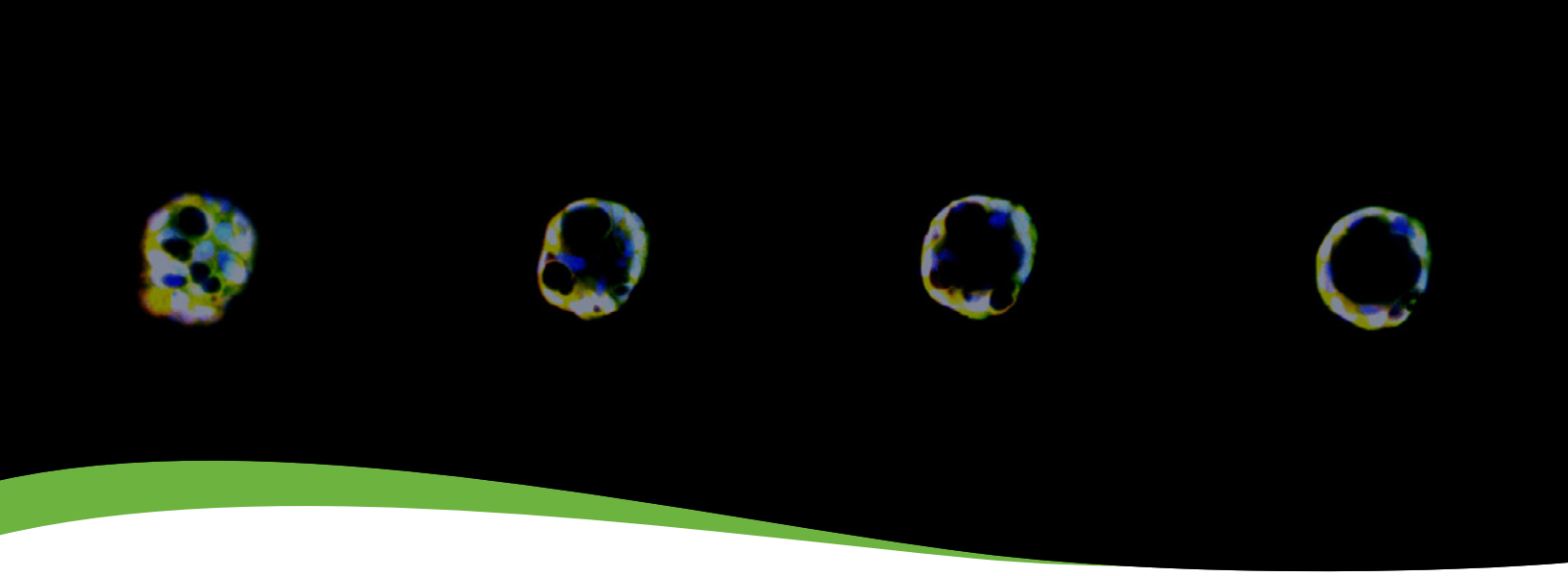


Using a high-content, confocal imaging system equipped with water immersion objectives and a high-intensity laser light source, scientists can improve image resolution and minimize aberrations so they can image deeper into thick samples.

Learn more about **how to leverage high-content imaging and analysis for your organoid workflow** here.



Technology 2



Technology

a. Confocal microscopy for 3D imaging

When using organoids for disease modeling and assessment of compound effects, the quality of images is important for downstream analysis. For maximum quantitative and robust assessment of phenotypic changes in organoids, as well as for increasing throughput of experiments and screens, high-performance, automated imaging and analysis solutions are of critical importance.

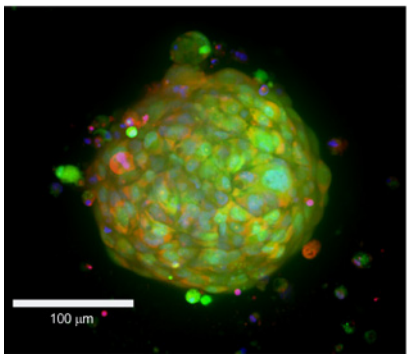
Specifically, high-throughput confocal imagers like the **ImageXpress® Confocal HT.ai High-Content Imaging System**, with high-performance lasers and water immersion objectives are particularly effective at capturing the complexity of organoid structures. The spinning disk confocal is especially suited for fast, optical sectioning which allows for 3D reconstruction of a sample from high-resolution stacks of images. The signal to noise ratio in the images is improved with the use of water immersion, and laser-based light sources allow for overall faster image acquisition.

Learn more about **the benefits of confocal imaging** here.

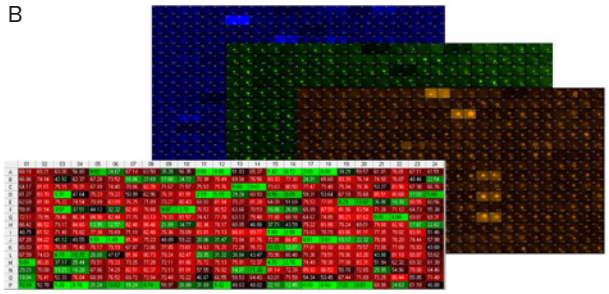
b. The benefits of high-content analysis

Organoids display complex cellular organization and structures and, as a result, can be characterized by various measurements. Individual cells, nuclei, or organelles can be fluorescently labeled and quantified within each organoid. This allows for live and dead cell analysis, specific marker quantification, and the ability to define volumes and distances between objects. Because not all images can be meaningfully or accurately analyzed with standard tools, **MetaXpress® High-Content Image Acquisition and Analysis Software** can customize image analysis needs to analyze spheroids, microtissues, cells in a 3D matrix, and small organisms.

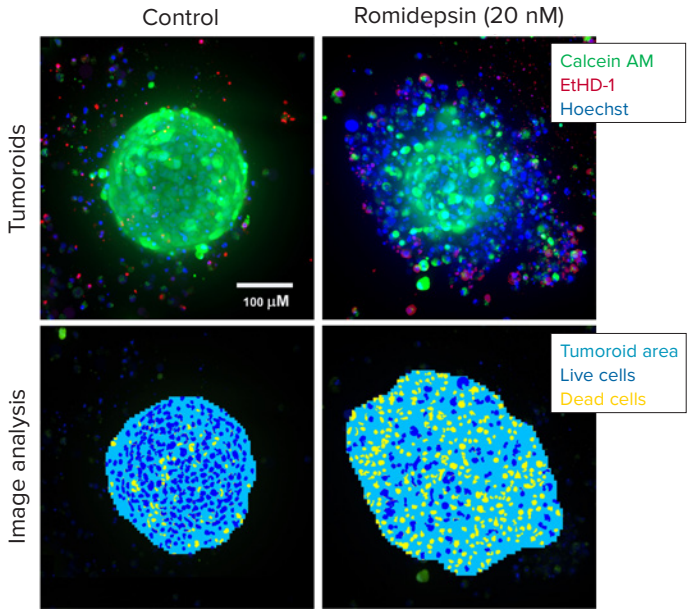
A



B



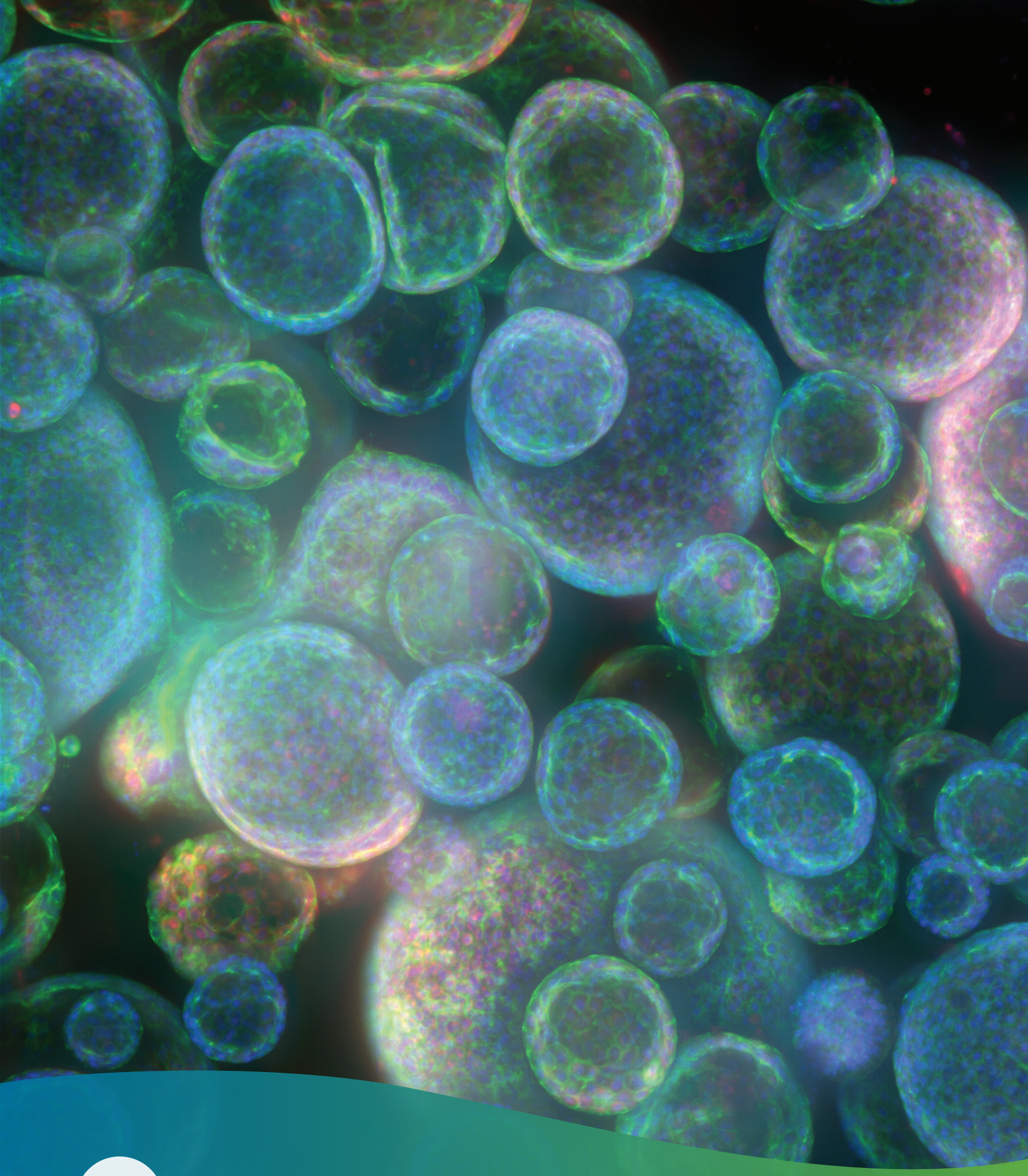
A. Tumoroid stained with E-cadherin (green), CD44 (red) and Hoechst, confocal image, 20X. B, Tumoroids were treated with compounds for five days then stained with Hoechst dye (blue), calcein AM (green) and EtHD (red), 10X. Organoids were imaged using confocal imaging.



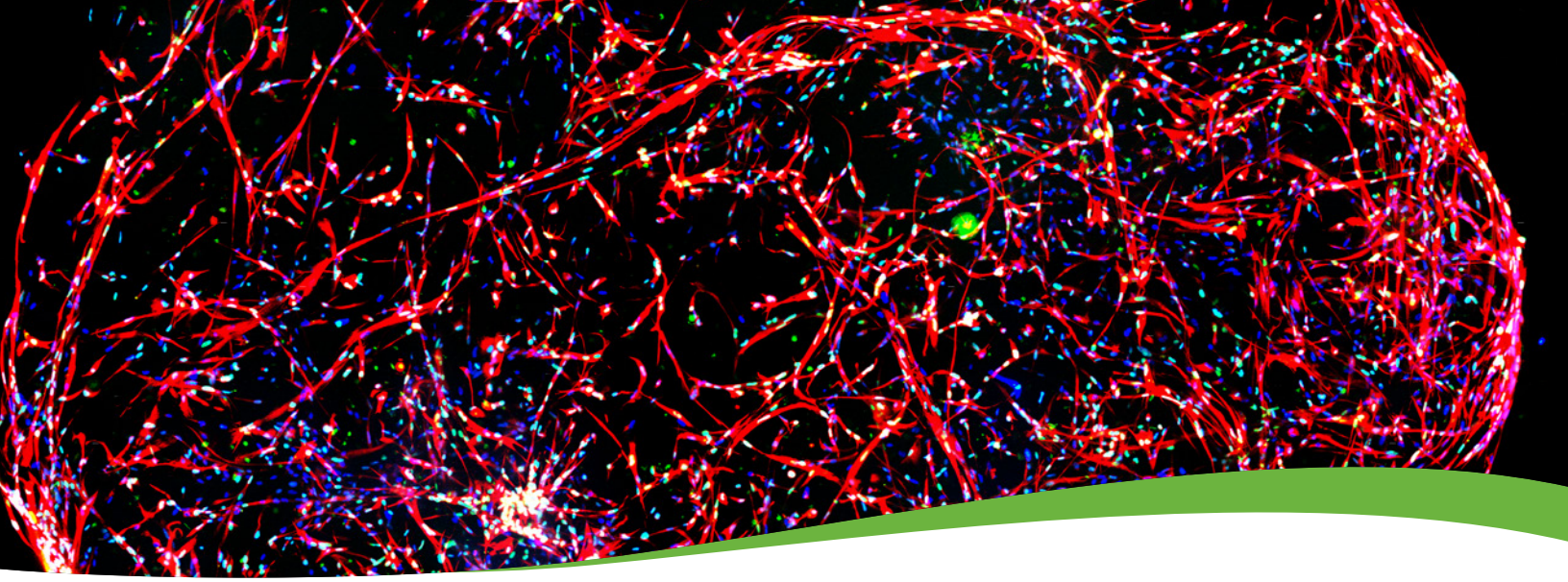
End-point analysis of fluorescent images was done using Custom Module Editor in ImageXpress software. Images and analysis masks shown. Multiple measurements were derived for cell scoring and organoid characterization.

Learn more about **the benefits of deep learning-based image analysis** here.

IN Carta® Image Analysis Software offers artificial intelligence (AI)-enabled tools for segmentation of biological structures with variable morphology or intensity profile, as well as imaging of uneven background or irrelevant debris. Then, phenotypic analysis can be performed using multivariate measurements to mine the data and categorize biological objects into different classes. Ultimately, AI-powered image analysis software, such as IN Carta, can solve complex high-content imaging problems and transform microscopy images into robust data. In addition, customization options allow researchers to train tailored deep-learning models, add post-analysis classifiers, and merge this data into efficient analysis pipelines for high-content imaging before visualization and review. By leveraging AI-based software to do the heavy lifting, scientists can focus on their research.



3 Applications



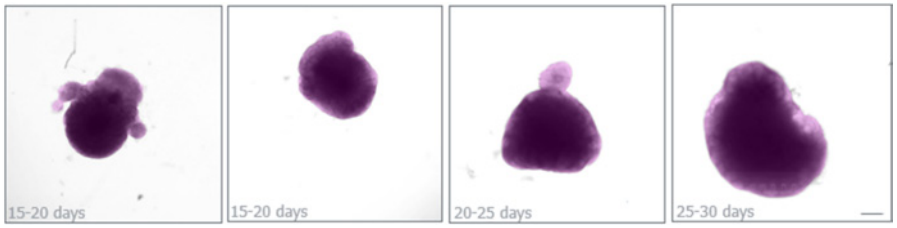
Applications

a. Brain organoids

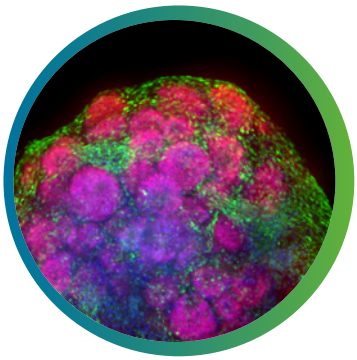
Brain organoids are 3D tissue models representing one or more regions of the brain. When cultured, human-induced pluripotent stem cells (hiPSCs) differentiate into various neural cells that mature over time to resemble structures of various brain regions, Cerebral 3D organoids are a rapidly developing technology that has great potential for understanding human brain development, neuronal diseases, and can be used for testing the effects of compounds and genetic mutations. This approach is highly promising for the evaluation of pharmaceutical drugs, studying effects of toxins, and in functional genomic applications.

Cerebral organoids are grown based on the Lancaster and Knoblich protocol (2014). The size and morphology of the developing brain microtissues were monitored using transmitted light microscopy over 20 weeks. An AI-based image analysis (IN Carta software) approach was used for defining the size, shape, and density of the tissues.

Selected microtissues were analyzed during the different phases of development using confocal imaging by the expression of Sox2, TuJ1, and GFAP markers.

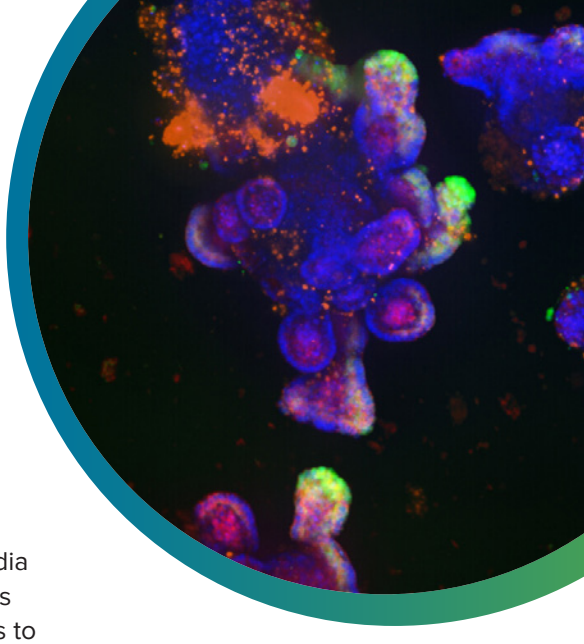


Analysis of brightfield images using deep learning-based segmentation (SINAP). Maturation of organoids can be monitored using brightfield imaging and analyzed using IN Carta (SINAP). Custom deep-learning model was trained and then applied to the dataset. Examples of organoids with segmentation mask (magenta) shown. Note that the SINAP model allows for segmentation of organoids with different shapes and sizes.



Cerebral organoids show organization similar to that of a developing brain. Organoids were fixed and stained with Hoechst (blue) and SOX2 (radial glia, in red). Shown here is one optical section from a four-week old organoid.

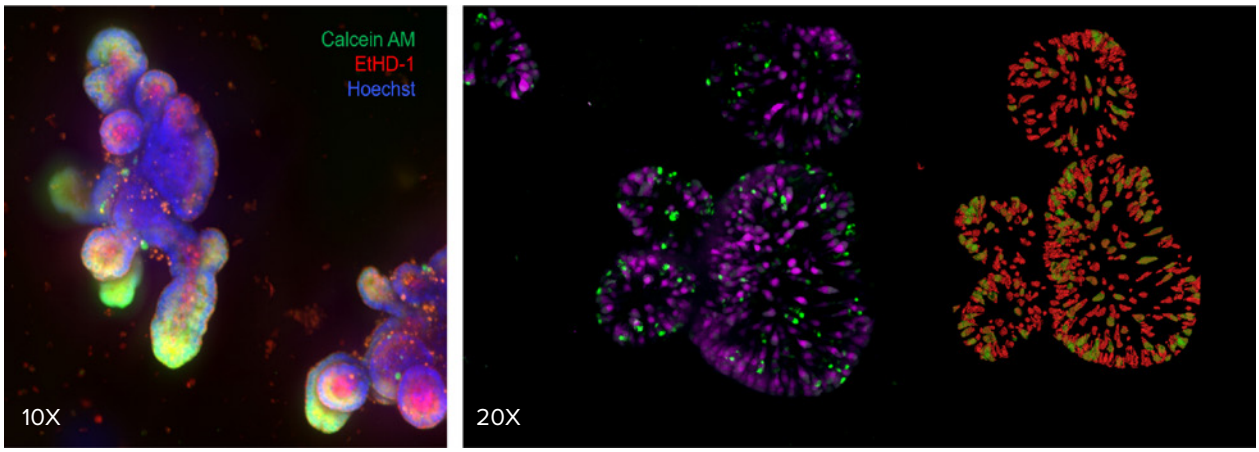
Learn more about **using machine learning image analysis tools to track the growth of organoids over time.**



b. Intestinal organoids

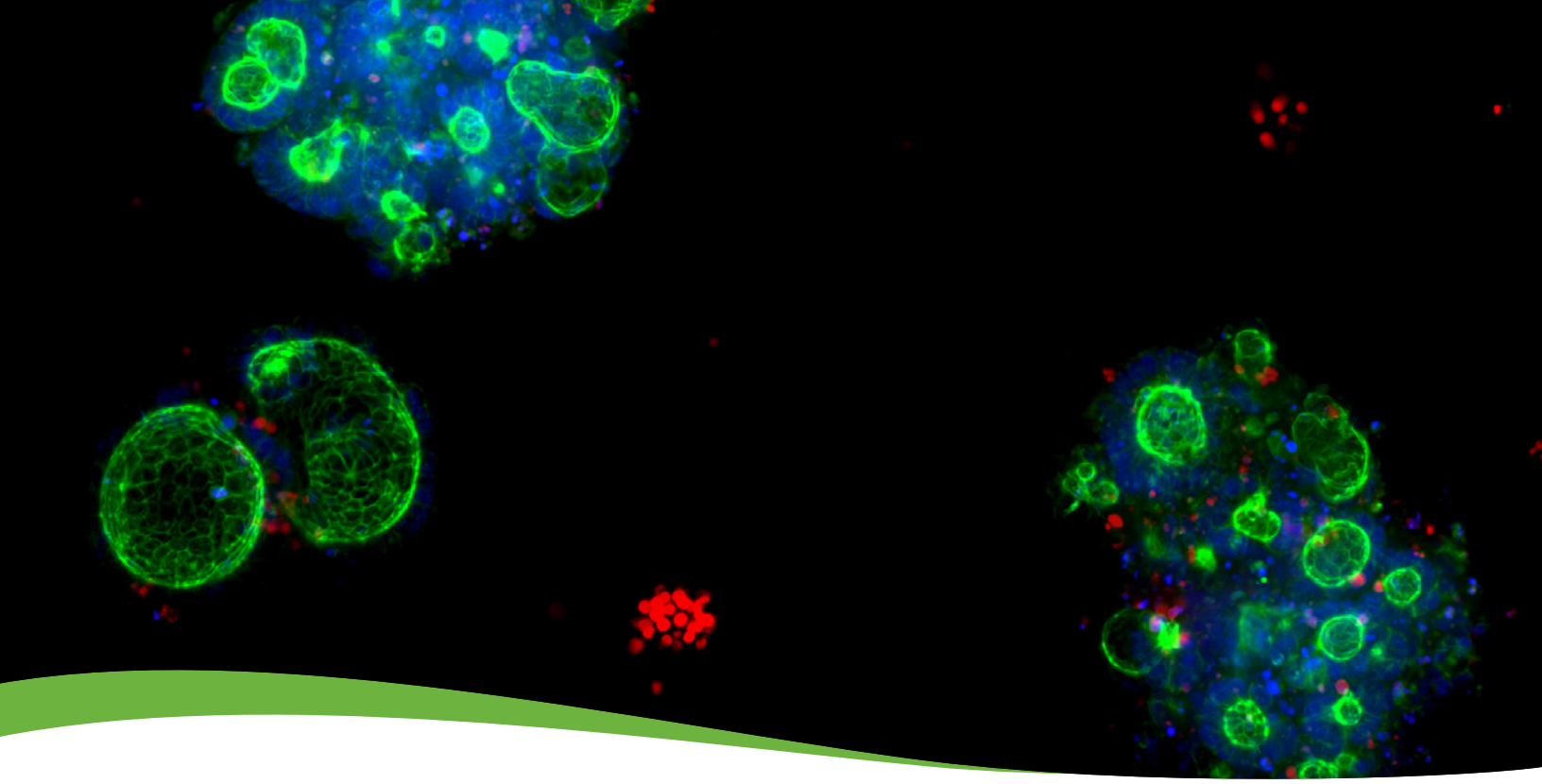
Intestinal organoids were one of the first 3D organoid models to recapitulate structures in the intestinal lumen and on the surrounding intestinal epithelium. The cell composition and arrangement of the epithelium make intestinal organoids useful for studying intestinal cell biology, regeneration, differentiation, as wells as disease phenotypes including effects of specific mutations, microbiome, or inflammation process.

In this application, 3D intestinal organoids were developed from primary mouse intestinal cells cultured in Matrigel. Methods for automated liquid handling were developed for cell seeding in Matrigel droplets and for media addition and exchanges. Six-week-old organoids were treated with various compounds followed by the live/dead assay and advanced image analysis to assess effects of treatment. In addition, methods to increase throughput, such as automated organoid assays and compound screening, are presented.



Organoids were treated and stained to detect dead cells and imaged using ImageXpress Confocal HT.ai system. Numbers of EtHD-1 positive (dead) and negative (live) cells were counted using 3D analysis and used to determine EC_{50}. Hoechst nuclear dye (blue) and EtHD-1 (red) and Calcein, AM (green).

Learn more about **automated systems to monitor, maintain, and characterize the growth of intestinal organoids.**



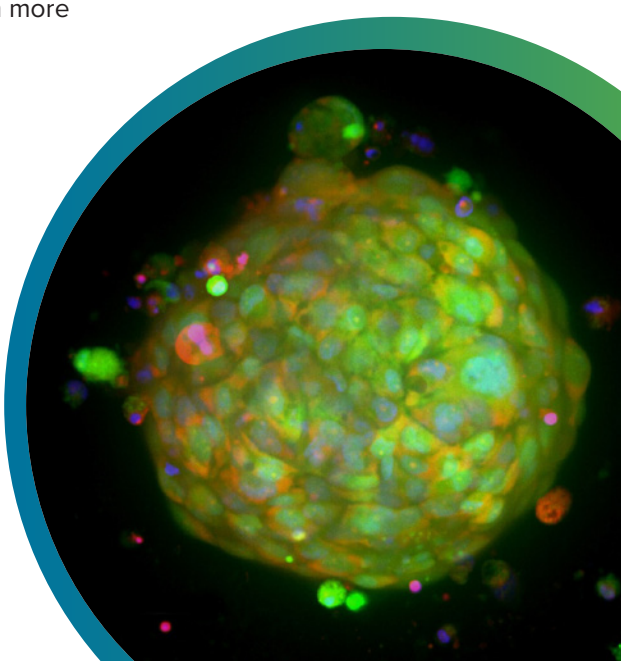
c. Patient-derived organoids (tumoroids)

Patient-derived organoids – or tumoroids – are 3D cultures that can be generated from primary tumors of individual patients. Tumoroids are highly valuable tools for cancer research, drug development, and personalized medicine.

For example, efficient cancer therapy is crucial in the survival of cancer patients. This necessitates the use of clinically-relevant tumor models to understand the biology of disease, analyze tumor biomarkers, screen for the most efficient anti-cancer drugs, and provide a platform to study responses to targeted therapies.

In this study, tumoroids were formed from primary cells isolated from a patient-derived tumor explant that represents metaplastic breast cancer with a triple-negative breast cancer subtype. Tumoroids were treated with compounds and monitored daily using transmitted light imaging. This was followed with machine learning-based image analysis that allowed characterization of tumoroids size, diameter, integrity, and optical density. End-point-assay tumoroids were stained with viability dyes and imaged. It also describes tools to increase throughput and automate 3D cancer assays and compound screening. Advanced analysis approaches and descriptors allow scientists to gain more information about complex cellular systems, disease phenotypes, and compound effects.

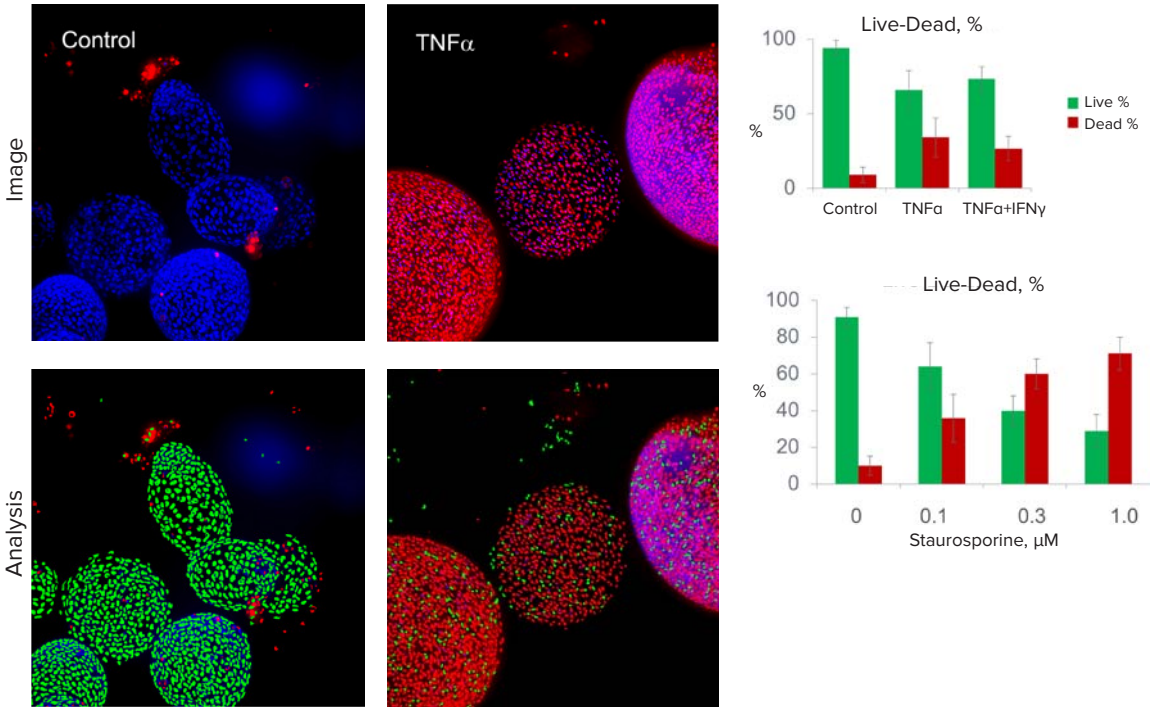
Learn more about **analyzing the growth and efficacy of anticancer treatments in breast cancer 3D tumoroid models.**



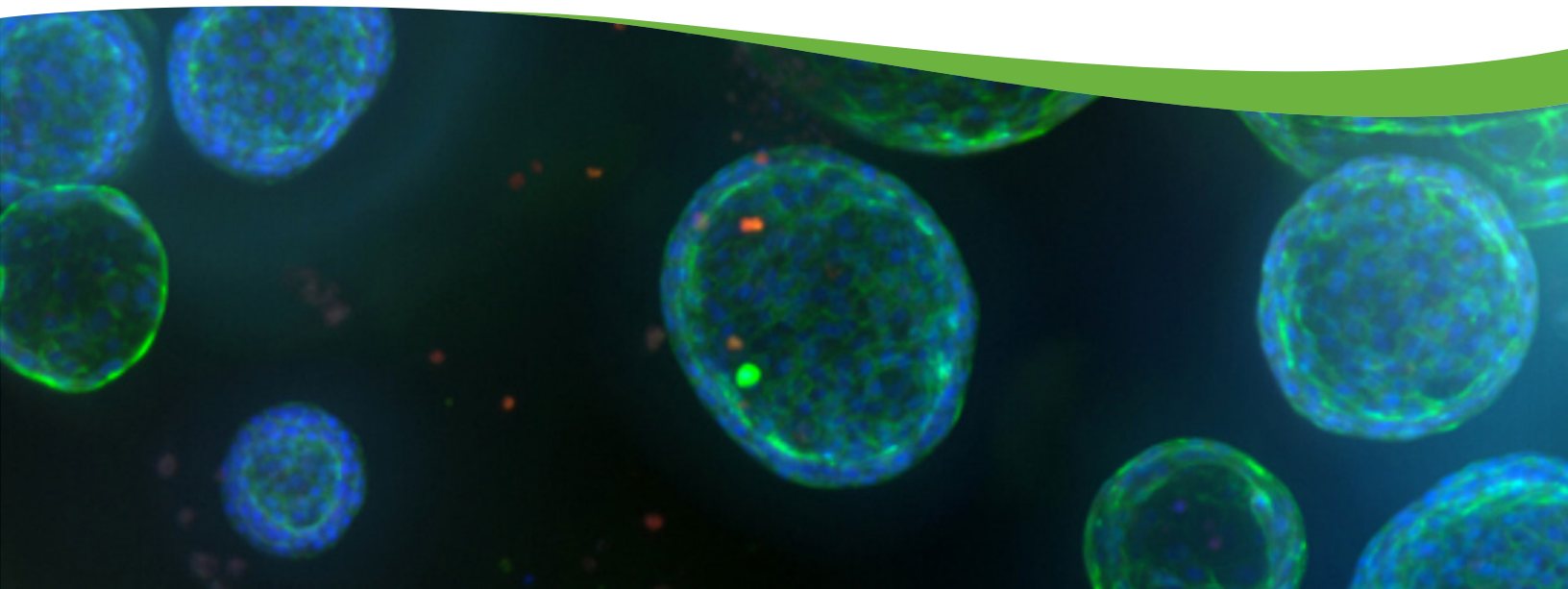
d. Pulmonary (lung) organoids

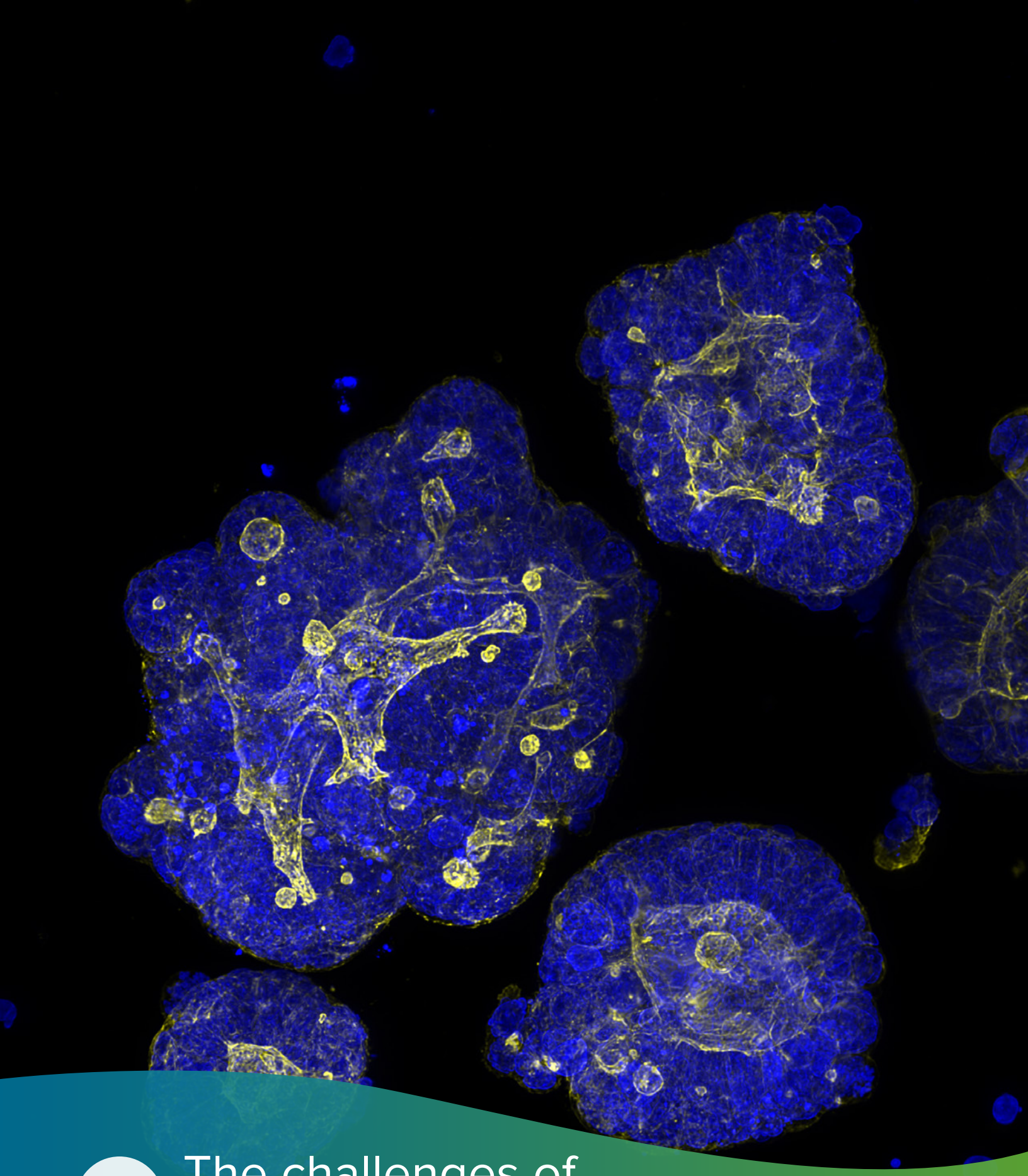
Lung organoid cultures are 3D microtissue models recapitulating the morphological and functional characteristics of the airway, which include alveolar structure, mucus secretion, ciliary beating, and regeneration. These special characteristics of the lung organoid culture hold potential for a wide range of applications in both basic and translational approaches such as drug screening and disease modeling.

In this application, primary human lung epithelial cells were cultured to model human lungs with the formation of 3D lung organoids. The cells within the organoids self-organize into complex structures that retain clusters of multi-lineage epithelial cells. High-content imaging was used to monitor the growth and differentiation of lung organoids. 3D reconstruction allowed for further complex analysis of the organoid structure. Within the organoids, the cell morphology, viability status, and the expression of various cellular markers can also be measured.



Learn more about **lung organoids for disease modeling and toxicity assessment.**



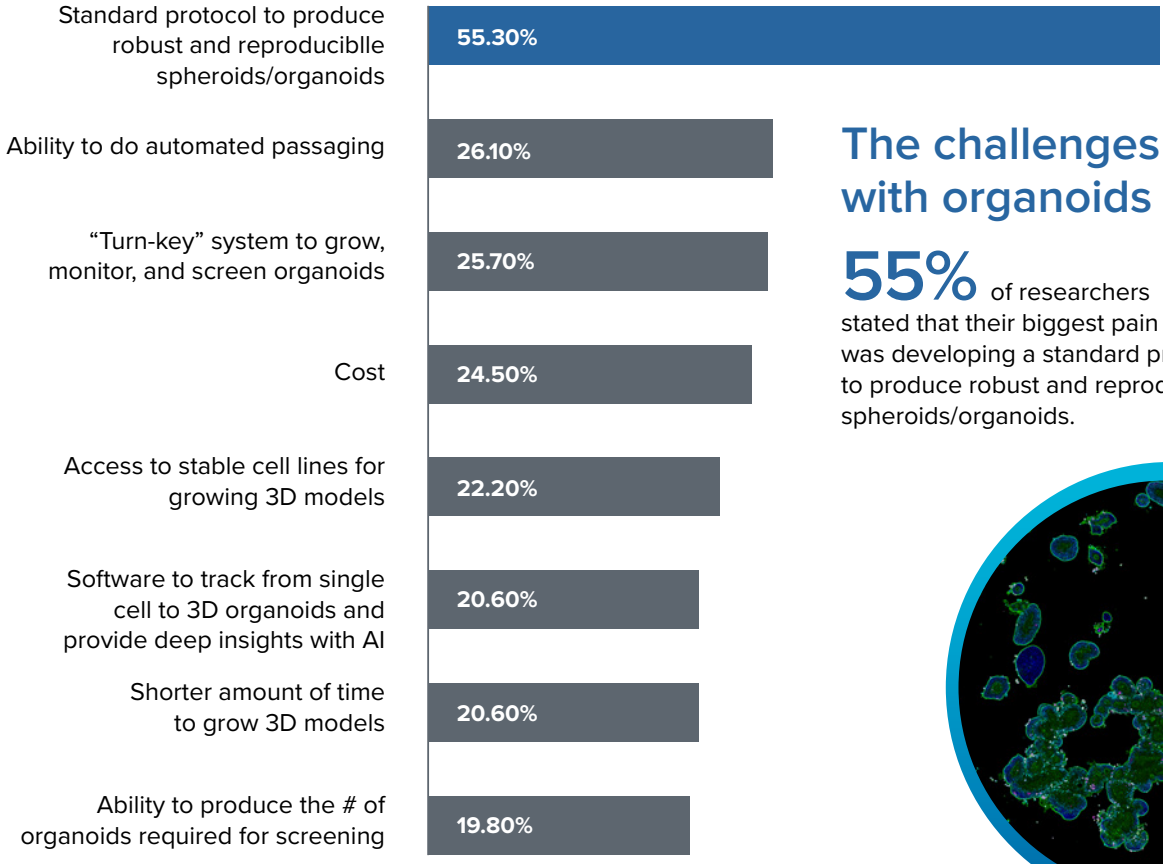


4 The challenges of using organoids at scale

The challenges of using organoids at scale

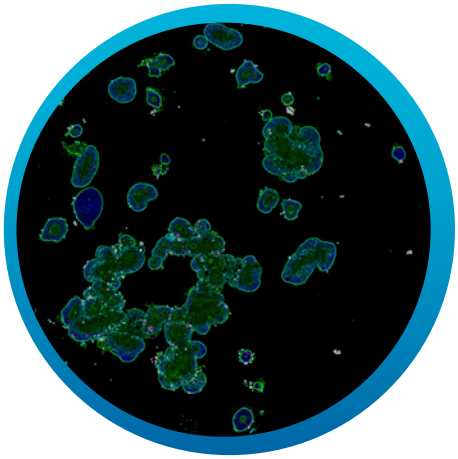
Obtaining reproducible, reliable organoids at scale

Traditionally, culturing and expanding organoids has been done manually. In addition to being technically challenging, time-consuming, inefficient and extremely labor-intensive, this method is not scalable and also results in inconsistently sized organoids. This has limited the suitability of using organoids in high-throughput applications, such as screening, and has hindered wider adoption of these models despite their relevance to human biology. Indeed, a majority of scientists in a recent survey tated that their biggest pain point when working with organoids was developing a standard protocol to produce robust and reproducible spheroids/organoids.



The challenges with organoids

55% of researchers stated that their biggest pain point was developing a standard protocol to produce robust and reproducible spheroids/organoids.



To meet these challenges, Molecular Devices has developed the 3D Ready Organoid Expansion Service, which facilitates large-scale organoid production from lines generated by our customers. These are returned cryopreserved to enable them to be thawed and used at the customers' convenience. Our semi-automated bioprocess, produces large batches of standardised, quality tested and uniformly sized organoids. Operator variability is reduced and the effects of inconsistent reagents and materials are minimised.

Organoids grown in our bioreactors can be used by both pharma and biotech companies in high-throughput assays such as compound screening or indeed any application requiring large numbers of easily-accessed, assay-ready organoids in repeatable batches.

Find out more about the **Organoid Expansion Service**

See how **expanded patient-derived organoids were used to evaluate chemotherapies**

Automating organoid screening

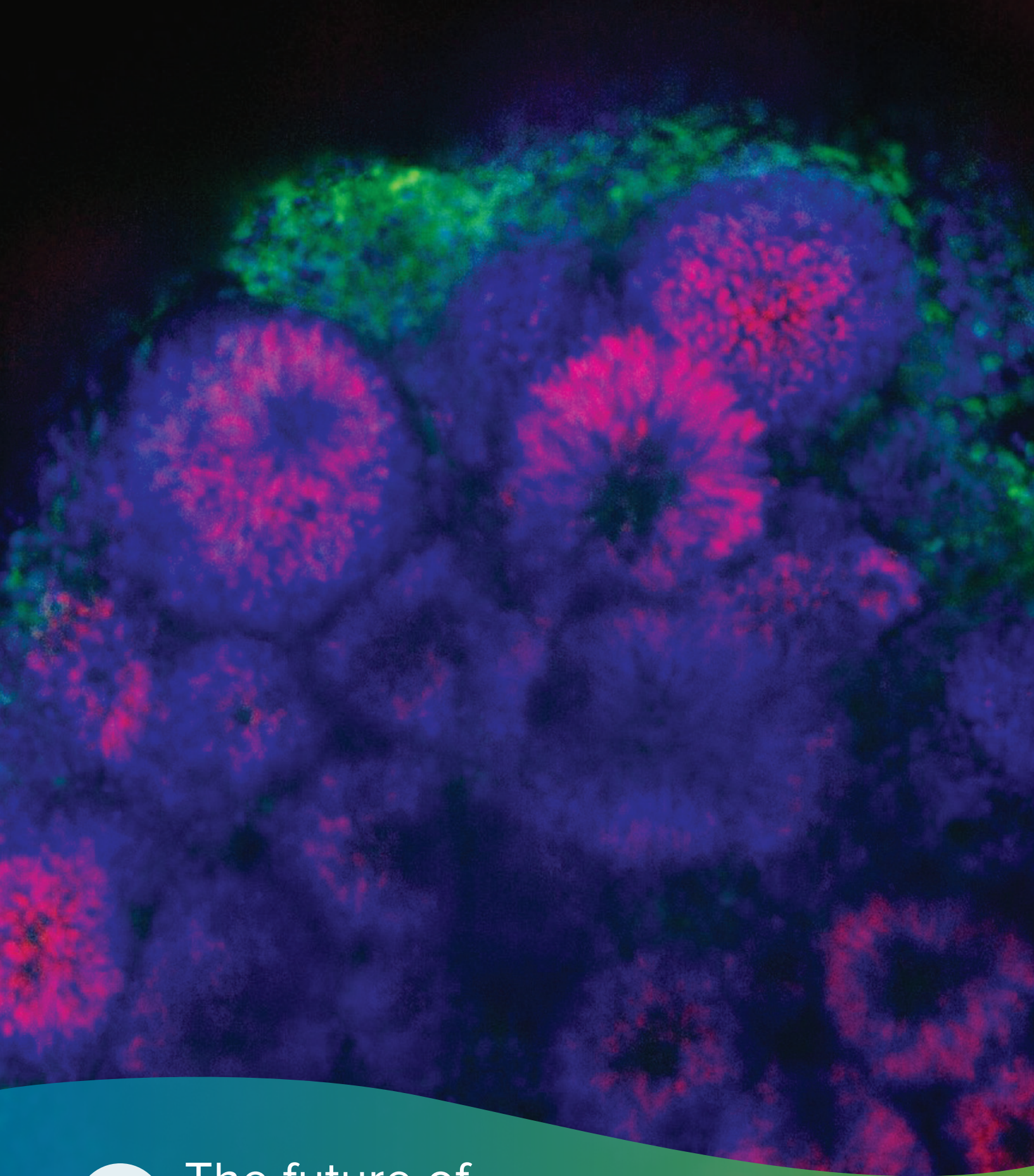
Another challenge faced when wishing to scale up organoid research is achieving higher throughput whilst delivering consistent, unbiased and biologically-relevant results at scale.

Automating the workflow can bring with it several advantages, offering improved scalability and reproducibility whilst minimizing manual intervention and streamlining the overall process.

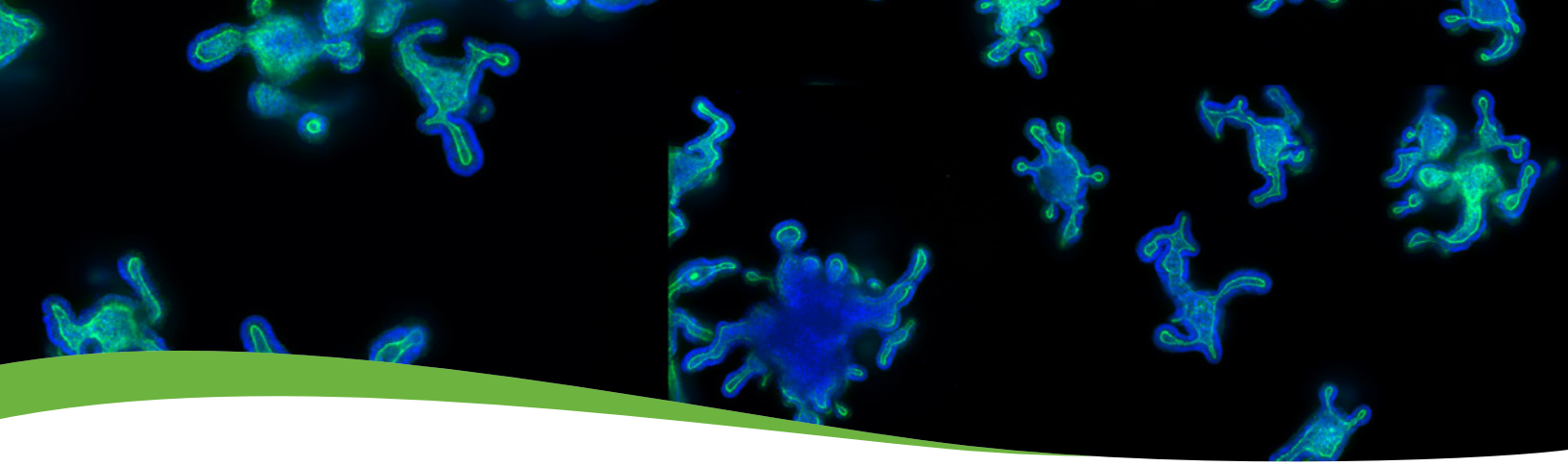
Our in-house scientists and engineers are experts in lab automation and can customize our instruments, as well as automate entire workflows to meet the specific needs of a researcher's assay, method, or protocol. From incubators, liquid handlers, and robotics to customized software and hardware—backed by more than 35 years of experience in the life science industry—scientists can count on us to deliver quality products and provide worldwide support.

Learn more about **how robotics-driven automation workcells and AI-based image analysis can help you develop an efficient, end-to-end workflow for the organoid development process.**

Take a **tour of our Organoid Innovation Center**



5 The future of organoid research

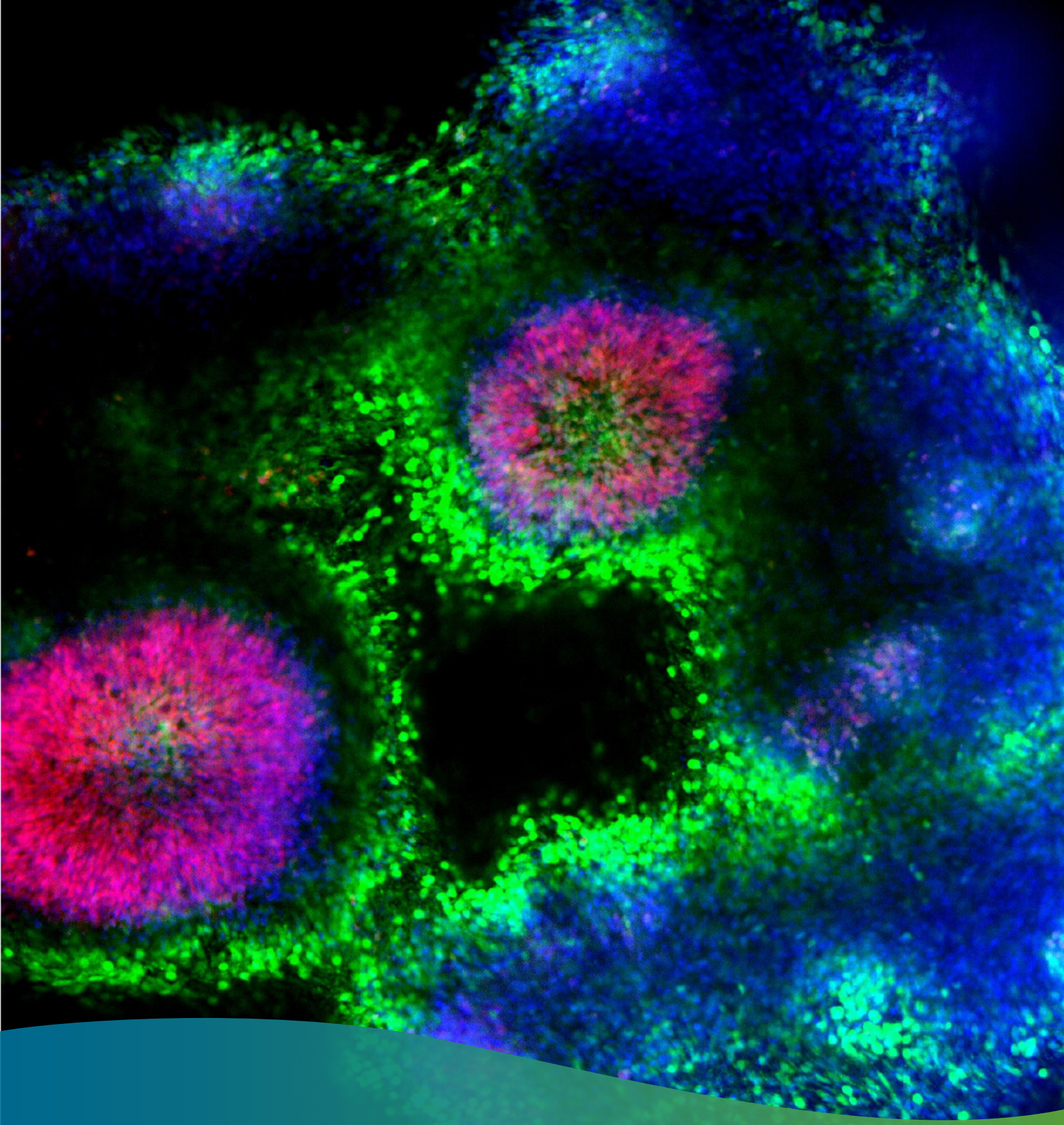


The future of organoid research

For three decades, Molecular Devices has made scientific breakthroughs possible for academic, pharmaceutical, government, and biotech customers with innovative life science technology. Looking to the future, our biopharma and 3D biology experts have compiled their life science and technology predictions for 2022, including:

- Enhancing clinical studies in drug discovery with AI and 3D models
- Predictive models for drug discovery
- Adoption of AI-based tools
- Development of precision medicine
- Personalized medicine
- Lab automation and intelligent screening tools for complex experiments
- A rise in 3D biology
- Innovative ways to get answers about COVID-19 and other viruses
- Advancements in sustainability and solutions to supply chain challenges

Read more about **their predictions** here



6 How researchers can start integrating organoids into their research

How do researchers get started integrating organoids into their research? *Here's a great place to start.*

Watch the webinar here.

View our webinar, **Getting started with 3D human tissue models and imaging** to get a general introduction to implementing 3D tissue models and imaging, and learn how to get powerful new insights quickly, easily, and affordably.

Watch the webinar here.

We recently hosted a webinar, **Capturing the complexity of 3D biology: Organoids for disease modeling and toxicity research** where we discussed how new methods and recent advancements in automated imaging and analysis are improving research in 3D biology. We describe automated cell cultures and a high-content imaging method that allow monitoring and characterization of growth and differentiation of intestinal and lung organoids–including toxicity effects.

References

1. Shamir ER, Ewald AJ. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. NLM. 2014. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4352326/
2. Lancaster MA, Knoblich JA. Generation of cerebral organoids from human pluripotent stem cells. NLM. 2014. https://pubmed.ncbi.nlm.nih.gov/25188634/

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