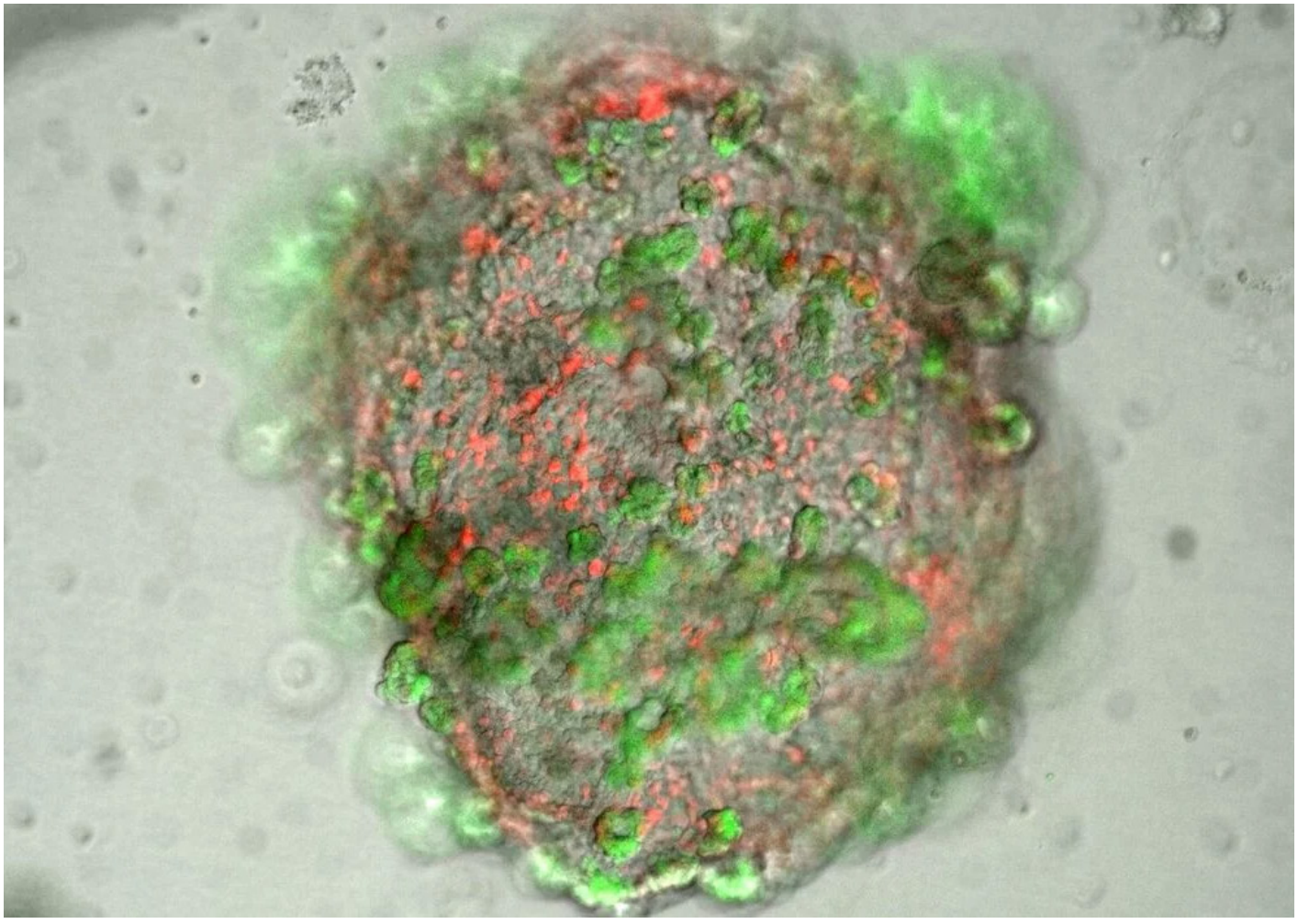
From Eye to Insight

Leica MICROSYSTEMS

OBSERVING 3D CELL CULTURES DURING DEVELOPMENT



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Abstract

3D cell cultures, such as organoids and spheroids, give insights into cells and their interactions with their microenvironment. These 3D cell cultures are playing an increasingly important role for researchers investigating novel cancer therapies, drugs to cure Alzheimer's disease, or personalized microfluidics to study chemotherapeutic efficiency in cancer patients. With a new imaging system, it is now easier to examine 3D cell cultures during development in real time at the cell level. For the microscopic imaging of three-dimensional samples, various options are available, such as confocal or light sheet microscopy.

Introduction

Old experimental techniques typically include imaging at the end of the workflow, as the organoids or spheroids would need to be removed from the cultivation environment. This manipulation of the cellular environment often resulted in a loss of sample material or malformation of 3D structure and was tedious. This meant that 3D cell cultures could only be observed when they had been finally developed. Imaging as an endpoint measurement on potentially damaged samples can be an excruciatingly frustrating experience. Handling a sample gently to retain its natural growth and cellular interactions is immensely important to understanding the mechanisms of disease. To observe 3D cell cultures during growth, the microscope has to adapt to the sample and provide an environment suitable for cultivation. In this case, imaging should not influence or interfere with further development. After imaging, the 3D cell culture should continue to develop. In addition to low mechanical stress during sample handling, this requires low light stress by using low light intensities and short exposure times.

With the THUNDER Imager systems, Leica Microsystems provides solutions that retains the favorable characteristics of widefield microscopes in handling, speed and low phototoxicity. The instruments further extend the range of application to three-dimensional samples. With conventional wide-field microscopes, a typical "turbidity" in thicker samples impairs the view of detail.

THUNDER imagers are equipped with the Computational Clearing technology. This innovative image processing technique effectively removes out-of-focus blur from areas outside the focal plane in real time. Computationally cleared images reveal the details of life which can then be easily identified, even at the depths of an intact living sample. By combining flexible illumination technology, high-resolution optics, stable environmental controls, and novel image processing techniques, THUNDER Imagers are intuitive, workflow-oriented, 3D cell culture solutions.

The therapeutic, regenerative potential of lung organoids

Research is being carried out to investigate the therapeutic potential of progenitor cells of different cell types in the case of acute and chronic lung diseases, such as influenza. Based on established transgenic mouse models, special cells are isolated and co-cultured (complex model system for the simultaneous cultivation of different cell types) with bone marrow-derived cells. The focus is on characterizing cell-cell communication at the molecular level, as well as transcriptional changes. The aim is to identify key molecules that cause transdifferentiation of pneumocytes from type 2 to type 1 (mainly responsible for gas exchange in the lung). This mechanism is often triggered by infections, but it is not yet understood in detail. In the long term, the research should make it possible to develop a personalized form of therapy tailored to help a patient after a lung infection, for example, and allows the damaged lung tissue to be regenerated.

The cultivation of lung organoids can be complex. Lung cells only generate at an air-liquid interface - similar to the human body. For this research, a 12-well plate holding nutrient medium and a porous membrane insert was used. On these inserts, the desired 3D cell cultures develop at the transition from matrix to air. Because of the different media and refractive indices, imaging is a challenge with this setup. It is not possible to remove the cells from the cultivation environment and return them for microscopic evaluation without damaging the valuable specimen. In the past, it was only possible to use a widefield microscope to check whether the fluorescent transgenes were expressed at all. Initial tests with a THUNDER Imager have already shown the formation of organoids down to the level of individual cells.

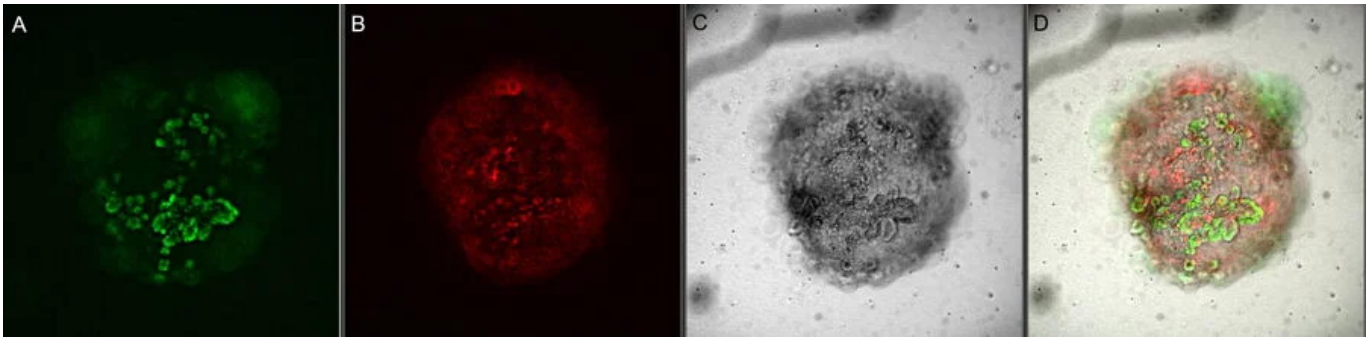

Figure 1: The images show a mature lung organoid taken at the "liquid-air interface" with a THUNDER Imager 3D Cell Culture. The cells originate from transgenic mice, so that the different fluorescence represents the degree of differentiation of the respective cell (A is YFP, B is mCherry, C is BF, and D is superposition). The image acquisition was performed on day 21 after the start of the culture. Reference: P. Kanrai, MPI-HLR Bad Nauheim.

Another important area of research focuses on a better understanding of the development and folding of the brain. To conduct this work, ferrets are used as model organisms. In contrast to the brain of mice, the much larger ferret brain has as many folds as the human brain and, therefore, is much better suited for these types of biological investigation due to its greater similarity to the human brain.

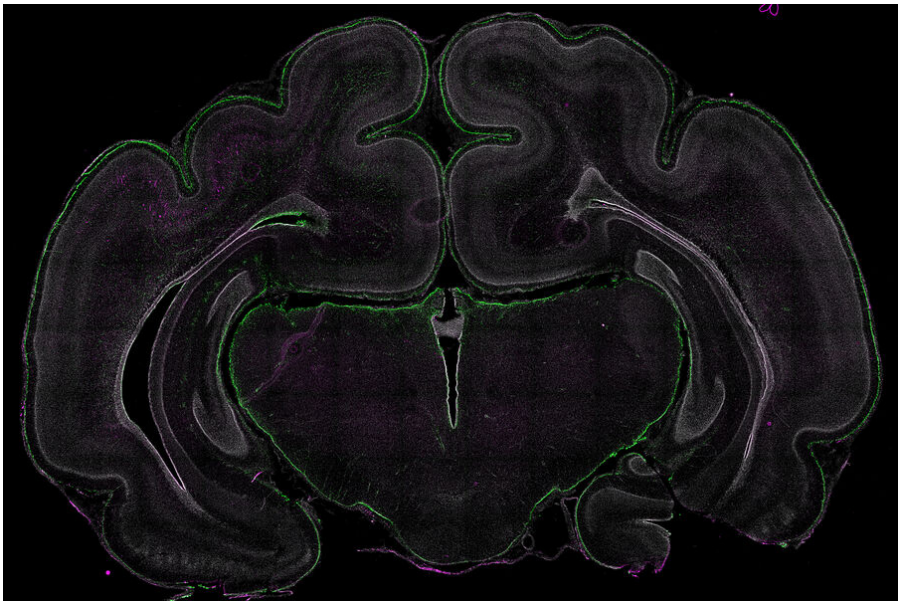

Figure 2: An overview of the developing ferret brain recorded on a THUNDER imager. Marked here are star-shaped glial cells (astrocytes in green) as well as the various developing neuron species (MCherry, magenta, marked using in utero electroporation). (B) Image shows a section with electroporated nerve cells (green), specific neuronal markers (magenta) and cell nuclei (white).

To understand normal brain development in ferrets, human genes were introduced into the ferret brain. Brains were genetically modified so that certain proteins can be localized during imaging. In the future, mutations could also be systematically investigated. The goal is to understand the patterns of brain folding which are large macrostructures. In order to be able to capture the entire sample context, the imaging focus is on a large tissue area. In order to quantify relevant parameters, individual cells must be recognizable within the same image. This requires a very large image field with sufficient resolution and clear imaging. In order to examine certain regions in detail on the subcellular level, confocal microscopy is used in a further step.

"The pictures of the THUNDER Imager are already after acquisition nearly ready for publication!"

Dr. Nereo Kalebic, Max Planck Institut - CBG Dresden

The size of a brain section at the stage of development that is relevant for this examination is approximately 2 cm2. For such large specimens, a THUNDER Imager with a large image field and clear image of the sections enables an efficient workflow. The resolution and contrast improvements provided by Computational Clearing allow cell nuclei to be distinguished easily and is the basis for meaningful and reliable results in terms of quantification. Until now, the same tissue sections had to be visualized with multiple imaging systems to do a proper post-processing of the images. Once acquired, THUNDERed images are almost ready for publication which is a great advantage, as researchers with less experience in image processing can quickly produce meaningful results.

The evolution of life science research from 2D to 3D cell culture has been an exciting progression that can enable significant improvements in the future for the treatment of human diseases. Thick 3D cultured cell specimens provide data with more physiological relevance in terms of gene expression and morphology. Organoids and spheroids allow the modelling of various physiological aspects: Development, homeostasis, regeneration, and disease. Organoid cultivation is on its way to becoming one of the most important tools for basic and applied research and has implications for future personalized medicine. Nevertheless, 3D approaches still face some challenges.

When creating and maintaining 3D cell cultures, it is a big advantage for scientists to have practical ways that enable them to image and analyze their results easily. The structure of organoids and spheroids can be difficult to image, so care must be taken to be gentle with the specimen. Leica Microsystems offers a portfolio of THUNDER Imagers, a new instrument class that provides a fast, gentle, accurate, and reproducible way to image organoids and spheroids. These imaging systems combine the speed and high-throughput capabilities of widefield microscopes with the power of Computational Clearing. Upright-, stereo-, and inverted-microscope-based THUNDER Imager systems allow intuitive imaging for various applications, such as neurospheres, lung organoids, or tumor spheroids. THUNDER Imagers provide an improved contrast and resolution in comparison to conventional widefield imaging systems, enabling researchers to gather insights into their research more efficiently and to expand their range of application.

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