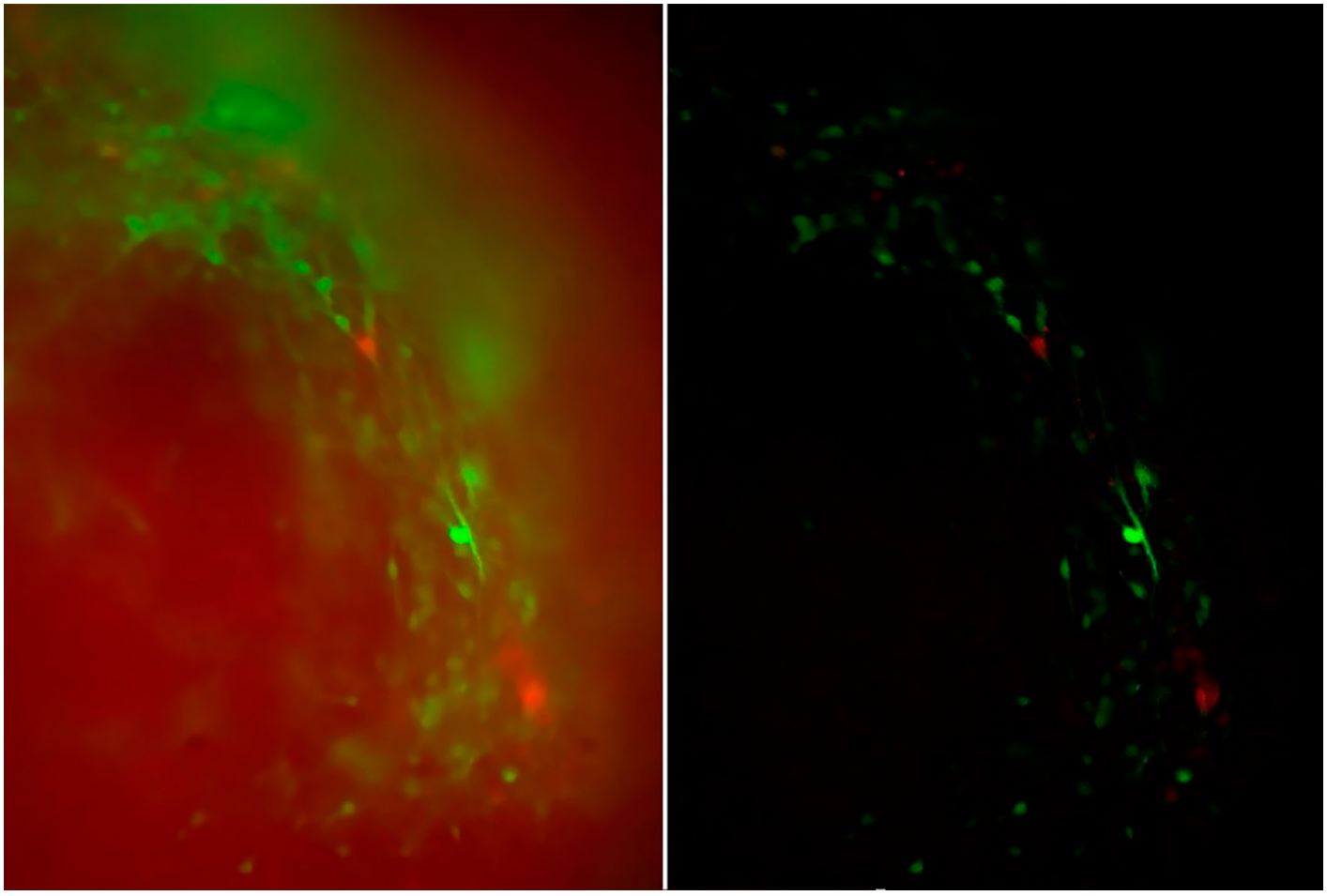
From Eye to Insight

“BRAINS-IN-A-DISH” FROM INDUCED PLURIPOTENT STEM CELLS (IPSCS)

Sharp, high-contrast imaging inside thick 3d cortical brain organoids



Authors

Samantha Lanjewar [1]
Steven Sloan [1]
James DeRose , Ph.D. [2]
Christopher Murphy [2]
David R. Barbero , PhD
[1] **Emory University, School of Medicine, Atlanta, Georgia, USA**
[2] **Leica Microsystems**

Abstract

This article discusses the benefits of using the THUNDER technology for imaging inside 3D human cortical brain organoids. These organoids are derived from human induced pluripotent stem cells (iPSCs) and can act as functional 3D "brains-in-a-dish" which can be used as models to study glia cell development and disorders like autism spectrum disorders. Imaging interactions between glia and neurons inside thick, large volume cortical organoids at high magnification and contrast requires fast multi-channel widefield fluorescence microscopy. A common problem of widefield microscopy is blur or haze when imaging inside thick specimens. Here, a Thunder Imager 3D Cell Culture was used in combination with Large Volume Computational Clearing (LVCC) to visualize clearly inside 3D organoids. The results show it can help facilitate the study of glial and neuronal interactions.

Introduction

Glia are essential constituents and regulators of the central nervous system (CNS) and are present in all organisms that have a CNS [1]. However, they differ considerably in diversity, function, and quantity. Model systems have contributed to improved understanding of glial-glial and neuron-glial interactions during CNS development and disease, but human glia exhibit specific attributes. Limited access to primary samples at critical developmental timepoints constrains the ability to assess glial contributions in human tissues [1]. This challenge has been addressed throughout the past decade via advancements in human stem cell differentiation protocols that now offer the ability to model human astrocytes, oligodendrocytes, and microglia.

Induced pluripotent stem cells (iPSC) derived from skin or blood cells can be re-programmed into an embryonic-like pluripotent state to provide a source of almost all somatic cells [2].

Non-invasively derived induced pluripotent stem cells (iPSCs) can be used to generate cortical brain organoids that act as functional 3D "brains-in-a-dish" and are useful as model systems for research [1]. Designed to resemble specific brain regions, these models allow the study of glial development and how this process may affect neurodevelopmental disorders, such as autism spectrum disorders [1]. Addressing these questions using in vitro systems requires imaging of multiple fluorescent channels for different cell types, through a large 3D volume, over long periods of time.

Challenges

To study thick biological specimens like organoids, it is most practical to have an imaging solution that allows fast screening over a large area of the specimen, but also enables higher magnification imaging with very good contrast at points deep inside it. Widefield fluorescence microscopy offers ease of use, speed, and detection sensitivity, but there are challenges when imaging thick specimens. Images of thick specimens often have "blur" or "haze" which significantly reduces contrast. This haze is produced by detected fluorescence signals which are emitted from out-of-focus planes in the specimen [3].

Methods

Human cortical organoids, 3D brains-in-a-dish, were generated from non-invasively derived induced pluripotent stem cells (iPSCs) [1]. The iPSCs were infected with the pAAV-hSyn-EGFP virus, which uses the human synapsin promoter to fluorescently label neurons (green), and the pLX-hGFAP-mCherry virus, which uses the human glial fibrillary acidic protein promoter to fluorescently label astrocytes (red) [1].

To image deep inside organoids approximately 3 mm thick, a THUNDER Imager 3D Cell Culture was used. To clear the images, they were processed with the Leica method large volume Computational Clearing (LVCC) [3].

Results

Images of a thick brain organoid derived from iPSC cells were recorded with a THUNDER Imager 3D Cell Culture and are shown below in figure 1.

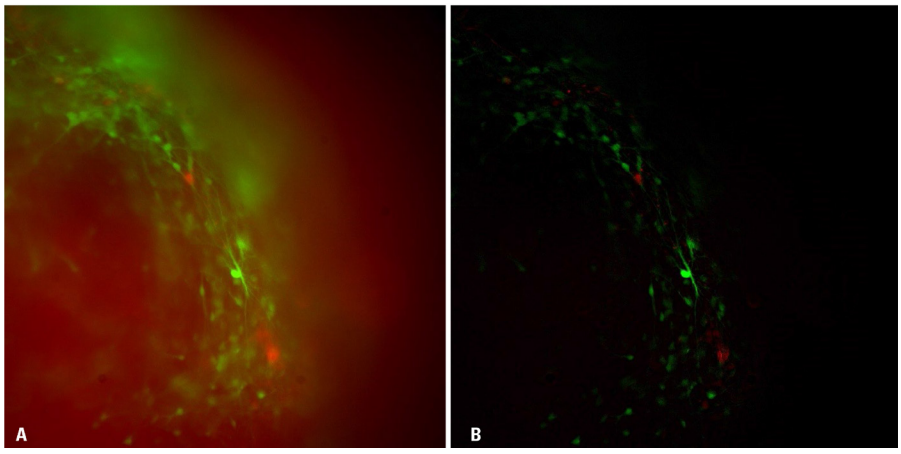


Figure 1. Images of a brain organoid derived from iPSCs acquired with a THUNDER Imager 3D Cell Culture. The cells were infected with the pAAV-hSyn-EGFP and pLX-hGFAP-mCherry virus. The image is the 36th plane cropped out of a 53 plane Z-stack volume. Shown are both the A) raw widefield image and B) the same image after Large Volume Computation Clearing (LVCC). Neurons are labeled in green and astrocytes in red.

Conclusion

The results showed that the THUNDER Imager 3D Cell Culture using Large Volume Computational Clearing (LVCC) [3] was capable of clearing the out-of-focus blur or haze from images of the thick cortical organoids. Being able to quickly and delicately image multiple fluorescence channels can help the study of interactions between glia and neurons as they develop together.

References

1. S.N. Lanjewar, S.A. Sloan, Growing Glia: Cultivating Human Stem Cell Models of Gliogenesis in Health and Disease, Front. Cell Dev. Biol. (2021) vol. 9, 649538, DOI: 10.3389/fcell.2021.649538.

2. T. Hamazaki, N. El Rouby, N.C. Fredette, K.E. Santostefano, N. Terada, Concise Review: Induced Pluripotent Stem Cell Research in the Era of Precision Medicine, Stem Cells (2017) vol. 35, iss. 3, pp. 545-550, DOI: 10.1002/stem.2570.

3. J. Schumacher, L. Bertrand, THUNDER Technology Note: THUNDER Imagers: How Do They Really Work? Science Lab (2019) Leica Microsystems.

MC-0002682 - 10.04.2024 - Copyright © 2024 Leica Microsystems CMS GmbH, Wetzlar, Germany. All rights reserved. Subject to modifications.
LEICA and the Leica Logo are registered trademarks of Leica Microsystems IR GmbH

CONNECT WITH US!

Leica Microsystems CMS GmbH | Ernst-Leitz-Strasse 17–37 | D-35578 Wetzlar (Germany)
Tel. +49 (0) 6441 29-0 | F +49 (0) 6441 29-2599

www.leica-microsystems.com/products/thunder-imaging-systems/