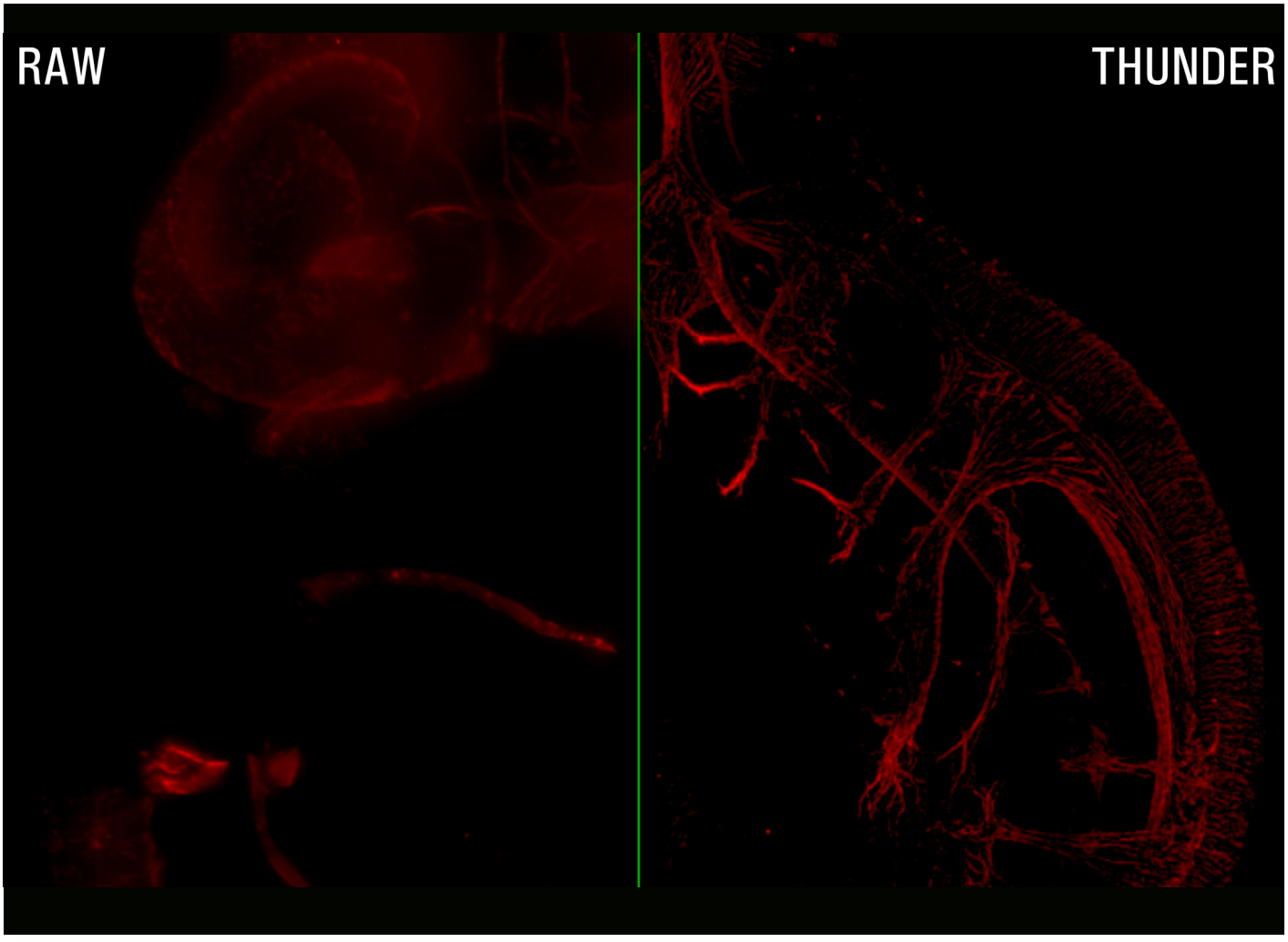
From Eye to Insight

Leica MICROSYSTEMS

CRANIAL NERVE DEVELOPMENT

Rapid, high-contrast imaging of mouse embryos to investigate axonal growth and pathfinding of cranial nerves



Authors

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Abstract

This article demonstrates how fast, high-contrast imaging of mouse embryos with a THUNDER Imager 3D Cell Culture and Large Volume Computational Clearing (LVCC) enable the investigation of axonal growth and cranial nerve development. Many genetic disorders which can compromise early stages in neural circuit development are believed to disrupt behavior. Studying cellular changes in early neural development with mouse model organisms and defining behaviors and underlying developmental mechanisms that meaningfully parallel human disorders is difficult. Identifying early divergence of axon growth in the developing-neuronal-circuit trigeminal nerve, which is involved in facial sensations and motor functions, allows these challenges to be addressed.

Introduction

Many genetic disorders are thought to disrupt behavior by compromising early steps in neural circuit development. Resolving such cellular changes in early neural development has proven challenging with model organisms. Defining behaviors, circuits, and underlying developmental mechanisms in mutant mouse models that meaningfully parallel clinically significant deficits in human genetic disorders is difficult. Detecting changes in initial differentiation of individual neurons remain elusive. These challenges are addressed by identifying early divergence of axon growth in a key component: the trigeminal nerve of the developing neural circuit. By focusing on the trigeminal nerve (cranial nerve V), which is responsible for sensation of the face and motor functions, such as suckling, feeding, biting, chewing, and swallowing, axonal growth and pathfinding in the native, 3D environment that might otherwise be missed using histological processing, can be can investigated. How rapid, high-contrast imaging of mouse embryos with a THUNDER Imager 3D Cell Culture and Large Volume Computational Clearing (LVCC) can help the study of cranial nerve development is shown this article.

Challenges

To image entire mouse embryos in a practical way, it is helpful to have a solution that can quickly achieve sharp, high-contrast 3D images where important details are clearly resolved. This way, the embryo can be captured in a single session and in a fraction of the time when compared to confocal imaging. Conventional widefield microscopy is fast and offers detection sensitivity, but images of thick specimens, like mouse embryos, often have an out-of-focus blur or haze which reduces the contrast.

Methods

Mouse embryos were imaged with a THUNDER Imager 3D Cell Culture. The embryos were stained with anti-βIII tubulin (Tuj1) antibody for mapping the nervous system and cranial nerves. Coupled with BABB clearing, the 3-dimensional structure of the nervous system in the entire embryo can be imaged. The images in figure 1 were collected with a 20x, multi-immersion media objective with a numerical aperture (NA) of 0.75 and a working distance close to 700 µm. The image consists of 32 stitched tiles with an imaging depth of 672 µm (337 steps), capturing the full extent of the embryo's structure. All data was collected in a total time of 18 minutes.

Results

The haze inherent to widefield imaging is extracted from the image via LVCC and Instant Computational Clearing (ICC). After clearing, the Leica adaptive deconvolution technology is used to enhance the resolution of the 3-dimenional features. This processing allows for easy viewing of the neural structure of the embryo as well as more precious placement of the neurons within the embryo's overall layout.

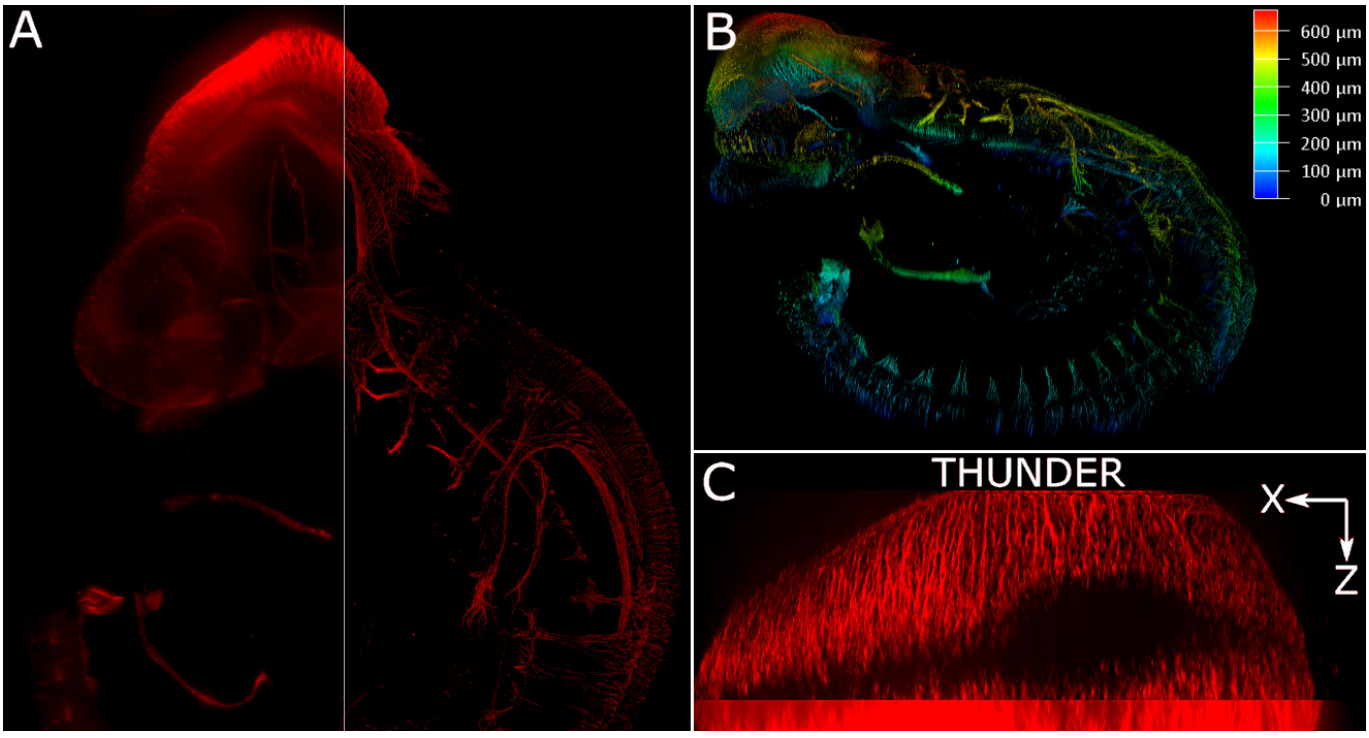

Figure 1: Top down view of whole mouse embryo illustrating the difference between the raw data before (A) and after (B) LVCC. Angled view of the embryo color coded according to depth relative to the objective where the maximum depth is 672 µm. C) Side view of the brain showing the fine detail revealed in the z-axis after applying LVCC. Image courtesy of Dr. Anastas Popratiloff and Dr. Zahra Motahari, The George Washington University Nanofabrication and Imaging Center (GWNIC), Washington, D.C., USA.

Conclusions

The THUNDER technology Large Volume Computational Clearing (LVCC) [2,3] significantly enhances the contrast when imaging cranial nerve development in mouse embryos allowing fine details to be resolved unlike conventional widefield imaging.

References

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