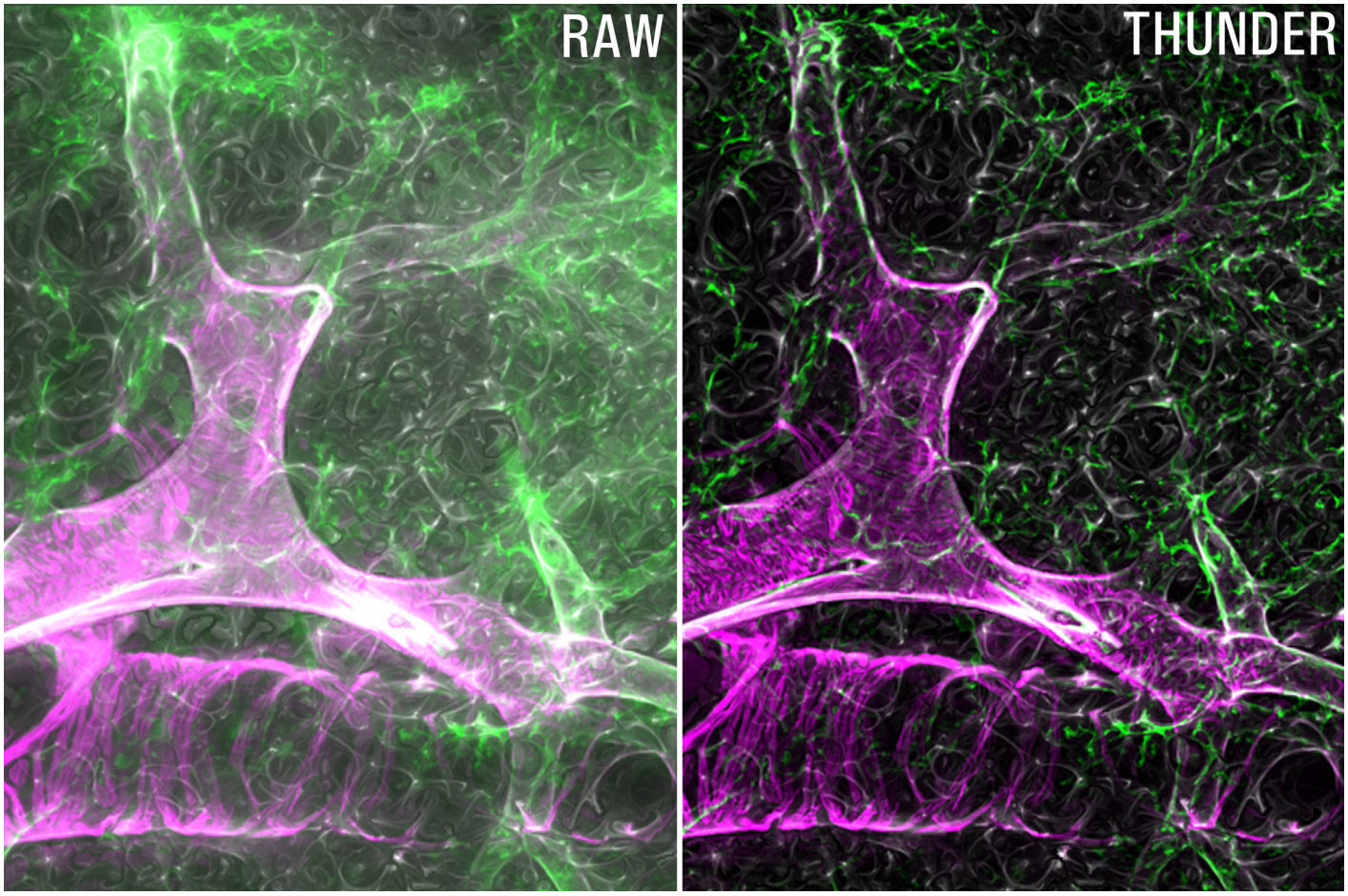
From Eye to Insight

Leica MICROSYSTEMS

INVESTIGATING PULMONARY VASCULAR DISEASES

Fast, high-contrast imaging of mouse lung to visualize the network of endothelia and supporting cells of the pulmonary vascular system



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Abstract

This article describes how the cellular and molecular programs governing the formation and maintenance of lung blood vessels, as well as what goes wrong with them when pulmonary vascular diseases occur, can be studied efficiently in mouse lung specimens with a THUNDER Imager 3D Cell Culture and Instant Computational Clearing (ICC). The pulmonary vascular system is a branched tubular network of endothelial cells, which line the vessels, and supporting cells that make up the vessel walls.

From a health perspective, it is interesting to identify the cell populations that give rise to these pulmonary blood vessels during development of the pulmonary arteries, veins, and capillaries, contribute to their maintenance and repair, and study the behaviors of these cells and the signals that control them.

Images of mouse lung: (left) raw widefield and (right) after the THUNDER technology instant computational clearing (ICC). Courtesy of Dr. Ross Metzger, CA, USA.

Introduction

The study of pulmonary vascular diseases involves various methods to probe cellular and molecular programs which have an impact on the diseases. Pulmonary vascular development begins in the embryo and continues after birth. The lung's branched tubular network consists of endothelial and supporting cells. To better understand vascular disease, scientists investigate the cells from which pulmonary blood vessels develop, as well as those that contribute to their repair.

Visualizing the network of endothelia and supporting cells' development and maintenance of the vascular system can be challenging due to the tubular structures along with the fact the lung specimens can easily be hundreds of micrometers thick. The results reported here demonstrate how mechanisms of pulmonary vascular disease can be studied effectively in mouse lung with a THUNDER Imager 3D Cell Culture.

Challenges

To obtain results practically when imaging lung tissue, it is helpful to have a solution that can quickly achieve sharp, high-contrast 3D images where important details are clearly resolved. Being able to image large areas of these thick specimens with conventional widefield microscopy, which is fast and offers detection sensitivity, results in a significant reduction in contrast due to the haze caused by out-of-focus fluorescent signals

Methods

Specimens of mouse lung were imaged with a THUNDER Imager 3D Cell Culture. The specimens were immuno-stained with FITC, Cy3, and Alexa 633. To visualize the whole lung specimen, a 20x Plan Fluo Apo 0.4 NA (numerical aperture) objective was used along with 3 fluorescent channels. The acquired images are displayed as Extended Depth of Field (EDoF) projections composed from 115 planes to cover the 280 µm thickness of the specimen.

Results

The THUNDER Imager 3D Cell Culture quickly acquired images of the large volumes presented by the lung specimen, then Instant Computational Clearing (ICC) was utilized to remove the haze that reduces contrast in the widefield image (refer to figure 1). ICC reveals in the lung images the fine structures and cellular resolution required to study the development, maintenance, and repair activities which impact pulmonary vascular diseases.

The entire volume acquired (see figure 1 below) was captured in about 1 minute.

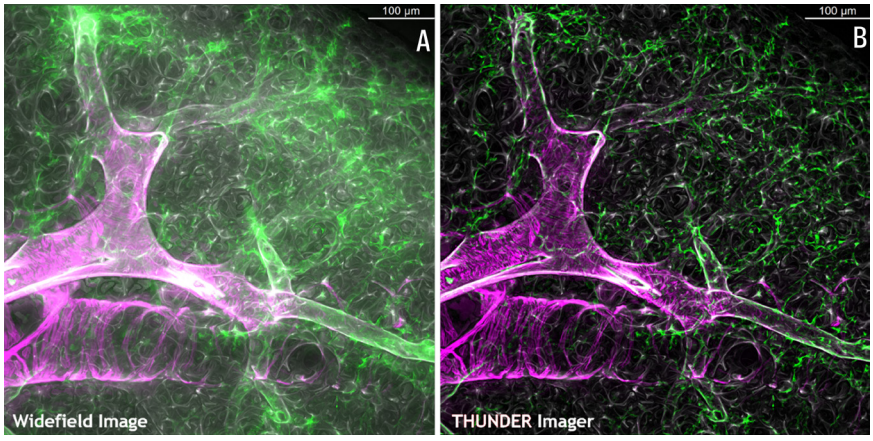

Fig. 1: Extended Depth of Field (EDoF) projections of the mouse lung specimen: A) Raw widefield image and B) after Instant Computational Clearing (ICC). Images courtesy of Dr. Ross Metzger, CA, USA.

Conclusions

An efficient study of the cellular mechanisms involved in pulmonary vascular disease using mouse lung specimens can be achieved with a THUNDER Imager using the technology Instant Computational Clearing (ICC) [3,4], because it significantly enhances image contrast compared to conventional widefield imaging.

References

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