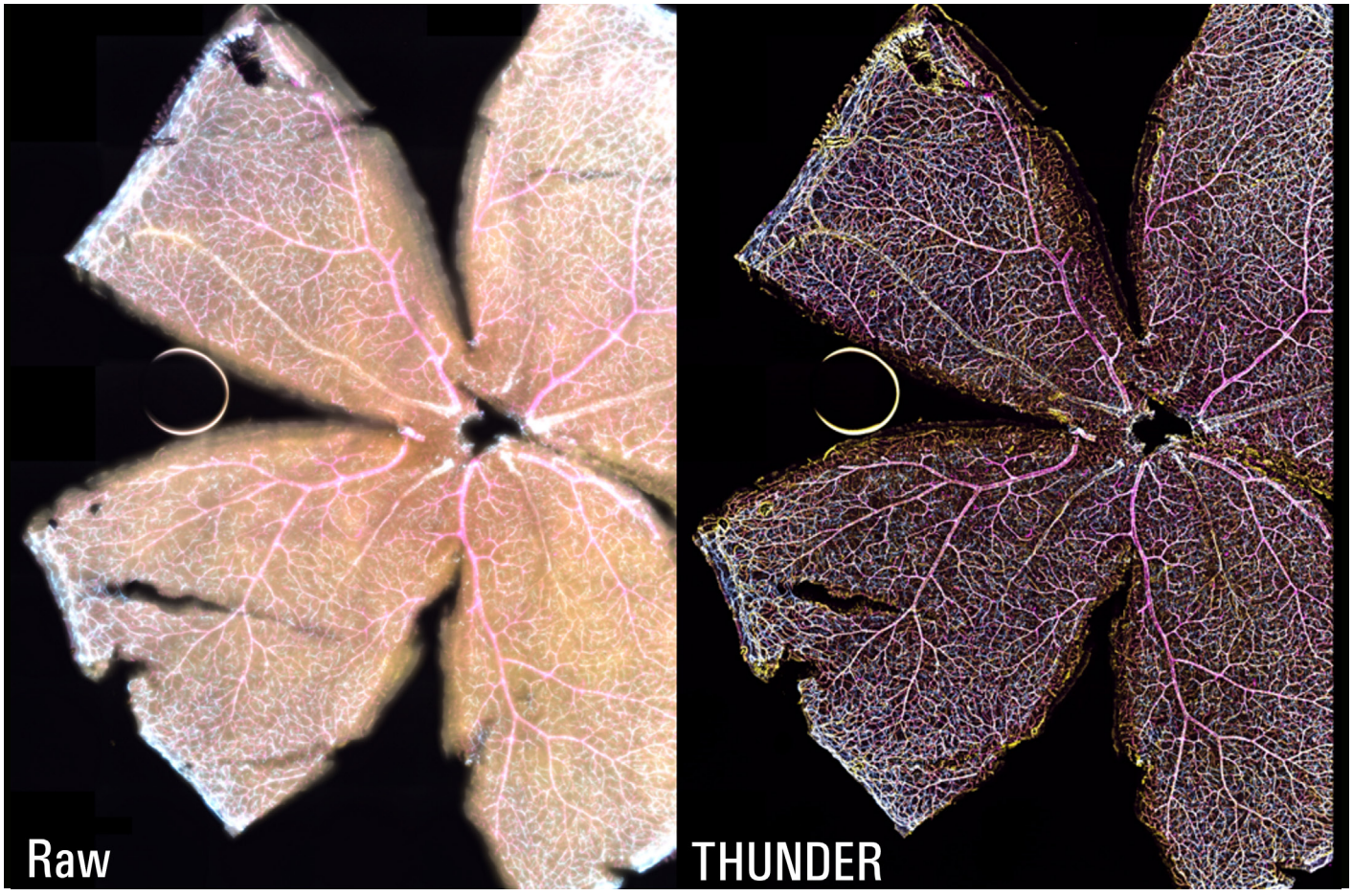
From Eye to Insight

Leica MICROSYSTEMS

VISUALIZING RETINAL INTERACTIONS TO STUDY EYE DISEASES

Rapid, sharp, high-contrast imaging of whole mouse retina to investigate interactions between endothelial cells, blood vessels, microglia, and astrocytes



Authors

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Abstract

This article shows how interactions between endothelial cells, blood vessels, microglia, and astrocytes in mouse retina can be studied efficiently with a THUNDER Imager 3D Cell Culture and Large Volume Computational Clearing (LVCC). Retinal vascular diseases are a major cause of impaired vision. Genetic mutations can cause alterations in the human retinal vasculature leading to diseases like familial exudative vitreoretinopathy (FEVR), Norrie disease, retinopathy of prematurity (ROP), or Coat's disease. Interactions between endothelial cells, blood vessels, microglia, and astrocytes in the retina can be investigated using mouse retina models. Whole-mount retina preparation and high-resolution fluorescence imaging of the whole retina can provide an overview of the vascular network and information on single cell interactions.

Introduction

Vascular diseases of the retina are a major cause of impaired vision and blindness. Retinas in the majority of mammals are perfused by three layers of vascular networks that include two intraretinal capillary beds. In humans, genetic mutations cause familial exudative vitreoretinopathy (FEVR), an inherited disease characterized by incomplete vascularization of the peripheral retina, Norrie disease, retinopathy of prematurity (ROP), or Coat's disease. The retinal vasculature is altered in each of these diseases. Scientists use the mouse retina model to study interactions between endothelial cells, blood vessels, microglia, and astrocytes which happen in the retina. The whole-mount retina preparation is exploited to visualize its vasculature Following fixation and staining, the whole retina must be imaged at high resolution to provide an overview of the whole vascular network, as well as single cell interactions. The results reported here demonstrate how interactions between cells in mouse retina can be studied efficiently with a THUNDER Imager 3D Cell Culture and Large Volume Computational Clearing (LVCC).

Challenges

To image whole retina 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. The retina imaging is typically done using tile scanning with a confocal microscope, but this approach takes several hours to acquire an entire image. Conventional widefield microscopy is fast and offers detection sensitivity, but unfortunately images of thick specimens, like whole retina, often show an out-of-focus blur or haze which reduces the contrast.

Methods

The whole-mount retina preparations were imaged with a THUNDER Imager 3D Cell Culture. The retina were labelled with anti-CD31 antibodies to indicate endothelial cells (yellow), IsoB4 for blood vessels and microglia (magenta), and anti-GFAP antibody for astrocytes (cyan). To visualize the whole retina, a 20x Plan Apo 0.8 NA (numerical aperture) objective was used in combination with a 100-position tile scan, a 15-µm Z-stack, and 3 fluorescent channels.

Results

The Large Volume Computational Clearing (LVCC) approach allowed a clear visualization of the interactions between endothelial cells, microglia, and astrocytes within the retina (see figure 1). Typically, these cells are difficult to discern in the retina with a widefield microscope. The high speed of acquisition with a THUNDER Imager 3D Cell Culture allowed a large tile scan, approximately 24 GB, to be acquired in minutes. The Focus Map approach with the LAS X Navigator software allowed the retina sample to remain in focus over large distances.

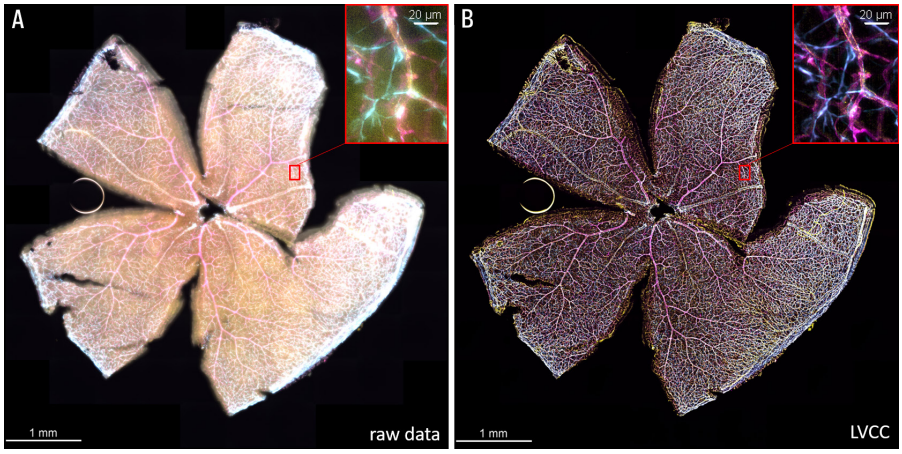

Figure 1: Maximum intensity projections of whole-mount mouse retina preparation imaged with a THUNDER Imager 3D Cell Culture: A) unprocessed widefield raw data and B) the results of LVCC. Insets show a magnified view of individual endothelial cells (yellow, anti-CD31 antibody), blood vessels and microglia (magenta, IsoB4), and astrocytes (cyan, anti-GFAP antibody). Image courtesy of Dr. Jiyeon Lee and Dr. Jeremy Burton, Roche Genentech, South San Francisco, CA, USA.

Conclusions

The THUNDER technology Large Volume Computational Clearing (LVCC) significantly enhances contrast when imaging whole mouse retina enabling an efficient study of interactions between endothelial cells, blood vessels, microglia, and astrocytes when compared to conventional widefield imaging.

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