From Eye to Insight

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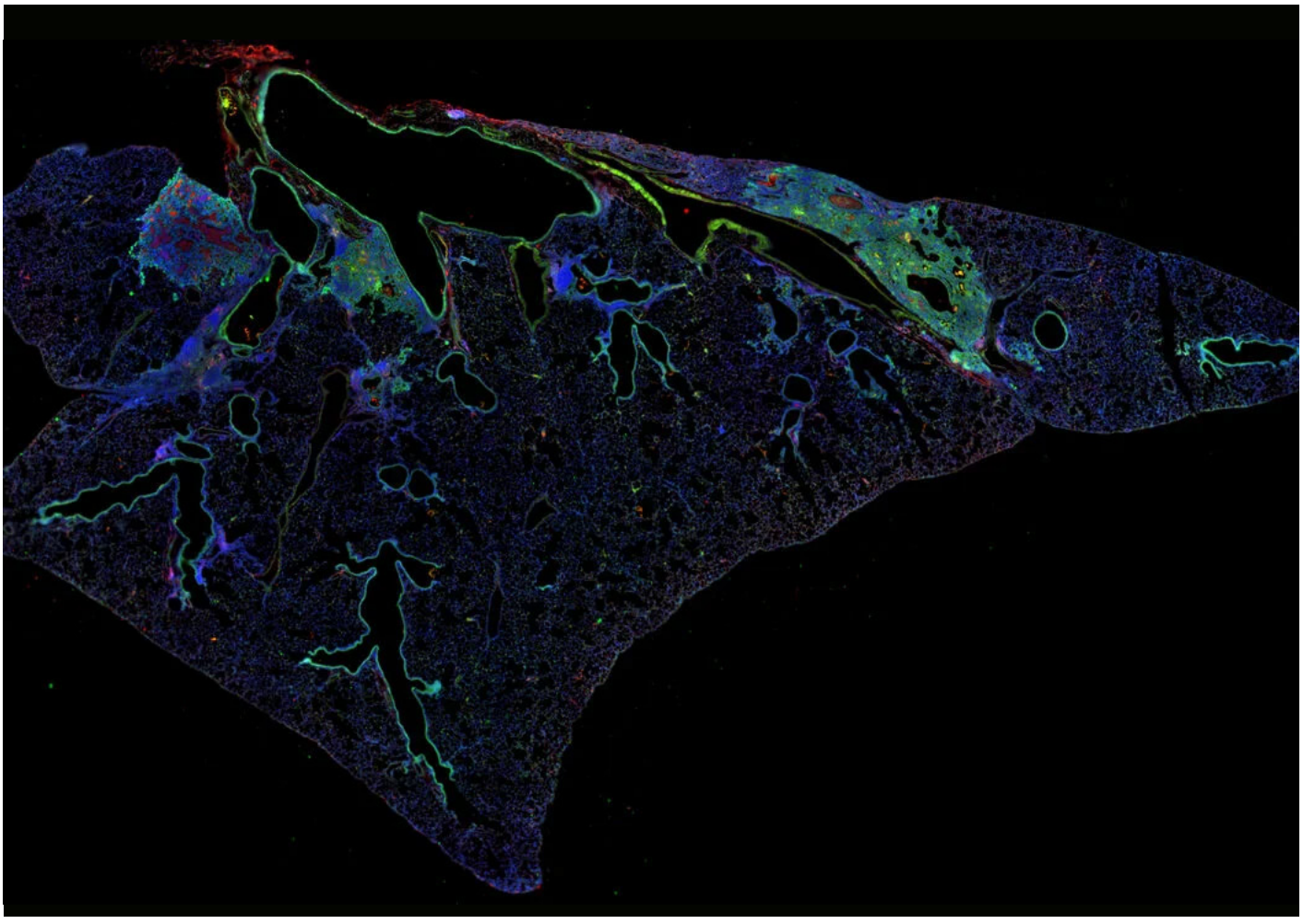
MONITORING IMMUNOSUPPRESSIVE MECHANISMS FROM INFECTION

Sharp, rapid imaging of lung epithelial tissues infected by an influenza virus



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Abstract

This application note discusses the importance of fast, sensitive, and haze-free imaging for the monitoring of immunosuppression in mouse lung epithelial tissue infected with the Puerto Rico 8 influenza virus. The study of how basal cell inhibition and immunosuppressive mechanism activation occur in virally infected lung tissue is critical for a better understanding of how viruses cause lung injury. Widefield microscopy offers fast-scan speeds and sensitivity, but lacks contrast due to a "blur" or "haze" when imaging inside thick tissues. However, sharp, haze-free images of lung tissue could be obtained efficiently using the THUNDER Imager 3D Cell Culture with Instant Computational Clearing (ICC). The THUNDER Imager can enable high contrast, high-throughput screening of lung tissue which would be practical for research on immunosuppression.

Introduction

Influenza virus infection can cause injury and damage to lung epithelial tissue. The extent of damage depends on the virus strain and host susceptibility. One goal of the research on lung injury due to viral infection is to better understand how the regression of lung basal-like structures are inhibited and immunosuppression is activated. For this study, the effect of the Puerto Rico 8 strain of influenza virus on mouse lung epithelium in terms of induced injury was investigated. Fluorescence microscopy was exploited to evaluate the initiation of basal cell expansion and activation of immunosuppressive mechanisms in lung tissue.

Challenges

For this type of research, it is important to have an imaging solution capable of fast screening of the lung tissue and detection sensitivity so that high throughput can be achieved, as speed of image acquisition is normally a bottleneck. The application also requires superior image quality to resolve structures of interest. To study thicker lung tissue sections, the solution should also image with good contrast at points deep inside the tissue. Widefield microscopy offers users speed and detection sensitivity, but when imaging thick tissue samples there is often a "blur" or "haze" due to signals emitted from out-of-focus planes in the specimen which reduces image contrast.

Methods

For this study, a mouse was used as the model organism and sections of mouse lung epithelial tissue were examined. Mice were inoculated with the Puerto Rico 8 strain of influenza virus to induce injury to the lung epithelium. Lung biopsies were collected two months after the viral infection. The lung tissue was immunofluorescently stained with Keratin 5, which is green and indicates basal cell expansion, and PDL1, which is red and indicates immunosuppressive mechanisms. Images of lung sections were acquired as high-speed, 3-channel tilescans using the THUNDER Imager 3D Cell Culture with Instant Computational Clearing (ICC) applied to remove the out-of-focus blur or haze. Fast scan speeds and sensitivity to achieve high throughput was important for this application, along with superior image quality to resolve structures of interest. To achieve these requirements, tile scans were taken with a 20x magnification, 0.8 numerical aperture (NA), plan apo objective lens.

Results

The THUNDER image below shows a section of mouse lung tissue. Such images enable markers for immunosuppressive mechanisms and regression of lung basal-like structures to be monitored.

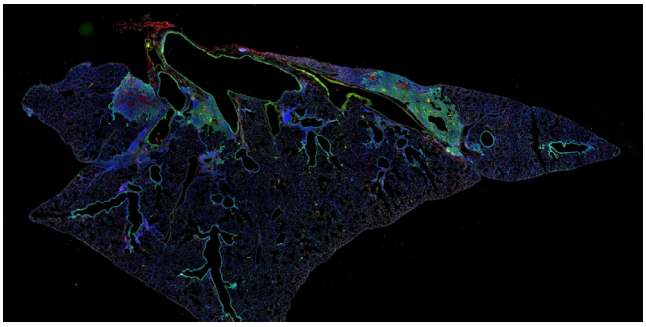

Fig. 1: THUNDER image of a section of mouse lung where the mouse was inoculated with the Puerto Rico 8 strain of influenza virus. The lung tissue was immunofluorescently stained with Keratin-5 (green) and PDL-1 (red). Image courtesy of Andrew Beppu, Stripp Lab, Cedars Sinai Medical Center, Los Angeles, USA.

Conclusions

The results show that the THUNDER Imager 3D Cell Culture using Instant Computational Clearing (ICC) is able to achieve fast screening and sharp imaging of lung tissue sections. Being able to quickly and clearly image tissue sections can help increase efficiency when studying how lung damage can be caused by influenza viral infection.

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