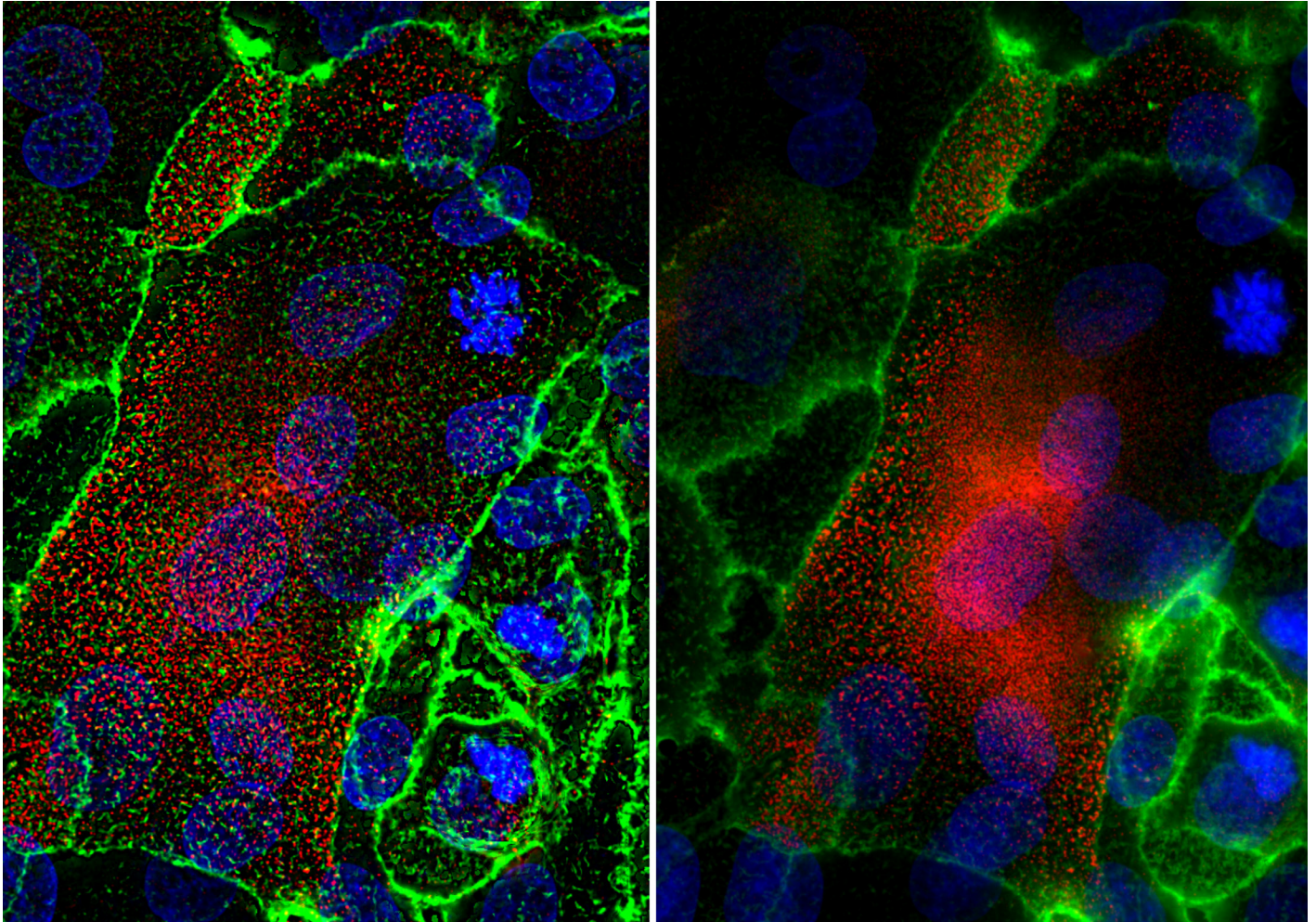


ROLE OF MUCINS AND GLYCOSYLATION IN DRY EYE DISEASE

High-contrast imaging of stratified human corneal epithelial cells



Authors

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Abstract

This article shows how fast, high-contrast, and sharp imaging of stratified human corneal epithelial cells with THUNDER imaging technology for dry eye disease (DED) research allows membrane ridges to be resolved. DED is common and can greatly affect the patient's quality of life for a prolonged time. The mucin proteins tethered on corneal epithelial cell membranes are hypothesized to provide barrier integrity and lubricate the eye. How mucins and their glycosylation factor into the pathogenesis of DED is not well understood due to a lack of direct and high-speed visualization methods. Sharp, high-contrast imaging of mucin layers has proven challenging with conventional widefield microscopy. However, this study demonstrates that membrane-tethered mucins expressed by corneal epithelial cells can be imaged with high-contrast using a THUNDER Imager Tissue and Large Volume Computational Clearing (LVCC).

Introduction

The mechanisms behind DED are not well understood. DED usually presents with mild discomfort. However, blurred vision and a reduction in quality of life can occur with prolonged disease. While DED is quite common, its pathogenesis still remains unclear. In particular, the role of mucin proteins and their level of glycosylation in the development and progression of DED is not well known. In the eye, transmembrane and soluble secreted mucins synergistically provide barrier and lubrication functions against environmental interrogations. The focus of this study is to visualize the presence of O-glycans on cell membranes which is characteristic of mammalian glycoproteins, such as mucin 1. Surprisingly, a high concentration of O-glycans was also observed on membrane ridges, supporting their potential role in epithelial barrier functions. In addition, mucin 1 is hypothesized to serve as a boundary lubricant, assisting blink motions. A dysfunction in either lubrication or barrier processes can potentially lead to DED. Here, human corneal epithelial cells were used to assess the level of glycosylated mucin 1 expression.

Challenges

When imaging mucins on the surface of stratified human corneal cells, confocal microscopy can be used to take z-stacks of the protein monolayers, but it can end up being quite time-consuming. A solution that can quickly image the mucins on the cell surfaces and achieve sharp, high-contrast 3D imaging, where important details are clearly resolved, is more practical. Conventional widefield microscopy is fast and offers detection sensitivity, but unfortunately images of thick specimens often show an out-of-focus blur or haze which reduces the contrast.

Methods

Stratified human corneal epithelial cells were fixed and immunofluorescently stained on day 3 with Hoechst (blue, marking DNA in the nucleus), Alexa fluorophore conjugated anti-bodies for mucin1 (red), and fluorescein-labeled jacalin lectin (green) [1]. Jacalin staining indicates the presence of O-glycans on cell membranes. The cells were imaged with a THUNDER Imager Tissue. A z-stack comprised of 44 slices representing a total thickness of 11.8 μm was taken using 3 channels in under 40 seconds (Figure 1). LVCC (Large Volume Computational Clearing) was applied to the image followed by an Extended Depth of Field (EDoF) projection.

Results

The use of THUNDER technology to optodigitally remove the out-of-focus blur resulted in the ability to resolve membrane ridges not seen when imaging with conventional widefield microscopy. The combination of LVCC and EDoF allows the details from all planes in one projection image to be clearly made out.

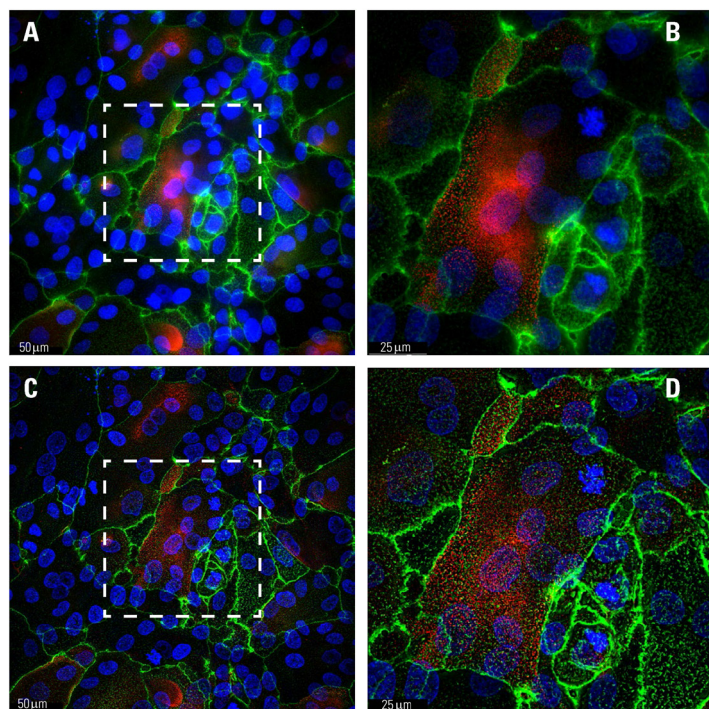


Figure 1: Images of stratified human corneal epithelial cells stained with Hoechst (blue), anti-bodies for Mucin1 (red), and jacalin (green). A-B) Raw widefield images and C-D) THUNDER processed, EDoF images. The white dashed-line box in 1A and 1C indicated the zoomed-in view of the cells seen in 1B and 1D.

Conclusions

The THUNDER technology Large Volume Computational Clearing (LVCC) significantly enhances the contrast when imaging human corneal epithelial cells, allowing the membrane ridges to be resolved which was not achieved with conventional widefield imaging.

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