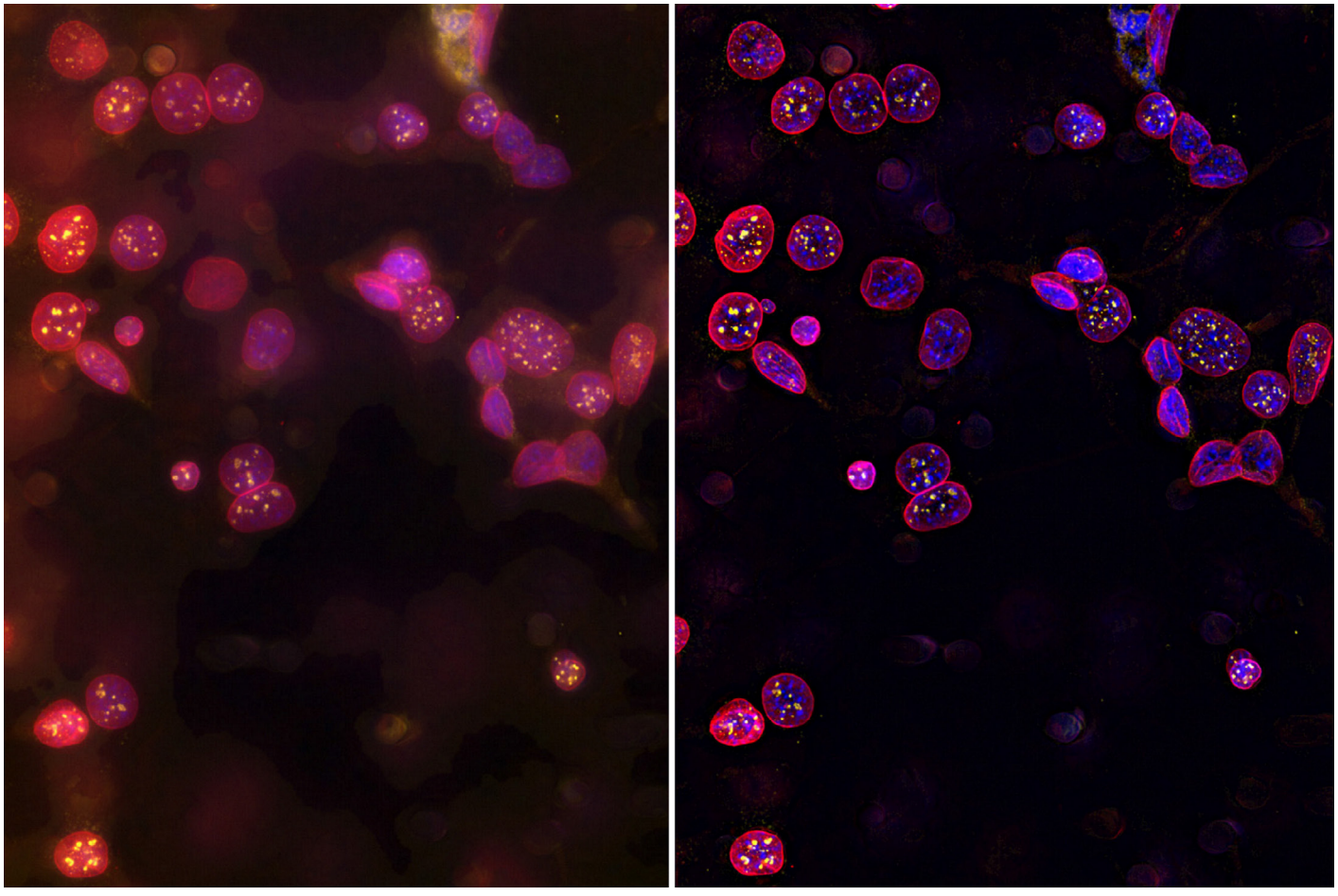
From Eye to Insight

SKELETAL MUSCLE ADAPTATION AND FIBROTIC DISEASES

High-contrast imaging of fluorescently labelled muscle cells to assess damage and regeneration



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Abstract

The mechanisms of how skeletal muscle adapts to fibrotic pathologies can be investigated more efficiently with fast, high-contrast imaging of C2C12 mouse myoblast cells which is described in this article. A better understanding of muscle fibrotic diseases and injured cell regeneration may lead to the development of novel therapies. Muscle regeneration studies require imaging solutions which can quickly screen muscle tissues and accurately assess cell damage. For this study, myoblast cells were labelled with a DNA-damage marker and then fluorescence imaging was done using a THUNDER Imager Live Cell with Large Volume Computational Clearing (LVCC). The quantification of DNA-marker foci, i.e., cell damage, was done more easily compared to conventional widefield microscopy.

Introduction

In order to make progress concerning muscle pathologies, like muscular dystrophies and cerebral palsy, it is important to gain a better understanding of the mechanisms by which skeletal muscle adapts, with an emphasis on the fibrotic process that is associated with many of these pathologies [1,2]. Researchers seek to uncover the relationship between the structure of the extracellular matrix and both the mechanical function of muscle and the regenerative capacity of muscle stem cells [3]. The hope is that these studies will lead to novel therapies for fibrotic muscle diseases. One of the main areas of research is to examine muscle cell regeneration after injury. To assess damage to muscle cells, they can be labeled with a DNA damage marker and imaged with fluorescence microscopy.

Challenges

For this muscle adaptation research, it is important to have an imaging solution that can quickly screen the cell culture specimens. It also requires high image quality so that the foci, marking DNA damage, can be clearly resolved. When imaging thicker specimens, the solution should also be capable of good contrast at points deep inside. Widefield microscopy offers users speed and detection sensitivity, but there is often an out-of-focus blur or haze, due to signals from out-of-focus planes of thick specimens, reducing significantly image contrast [4]

Methods

For this study, C2C12 mouse myoblast cells [3], precursors for muscle fiber, were examined. The cells were grown and migrated onto both sides of a transwell membrane and stained with lamin B (magenta) to label nuclear structures, Hoechst (blue) to label DNA, and yH2AX (yellow) to indicate DNA damage. Damage to cells following 3D migration through small constrictive pores was assessed by counting foci. Fluorescence images of the cells were acquired using a THUNDER Imager Live Cell with a 63X/1.4 NA (numerical aperture) oil immersion objective. Large Volume Computational Clearing (LVCC), which consists of Instant Computational Clearing (ICC) followed by Adaptive Decision Mask Deconvolution [4], was applied to remove the haze.

Results

The images below show the C2C12 cells. The images represent extended depth of field projections of 17.47 µm thick z-stacks.

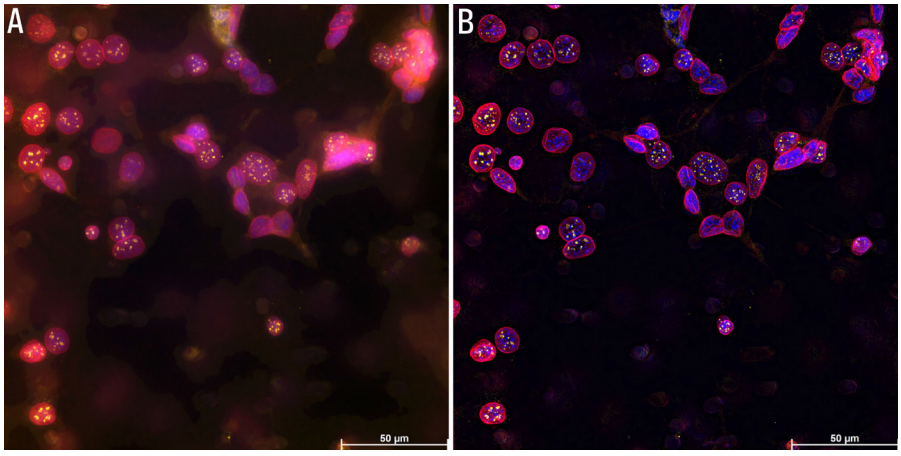

Figure 1. Images of C2C12 cells which were stained with lamin B (magenta), Hoechst (blue), and γH2AX (yellow). A) raw widefield fluorescence image and B) THUNDER image after LVCC. The yellow foci indicate damaged DNA.

Conclusion

The results show that when using conventional widefield fluorescence microscopy, the images are hazy. However, after applying THUNDER Large Volume Computational Clearing [4], the contrast is significantly enhanced. The THUNDER Imager allows for easier automated quantification and counting of foci compared to conventional widefield microscopy.

References

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