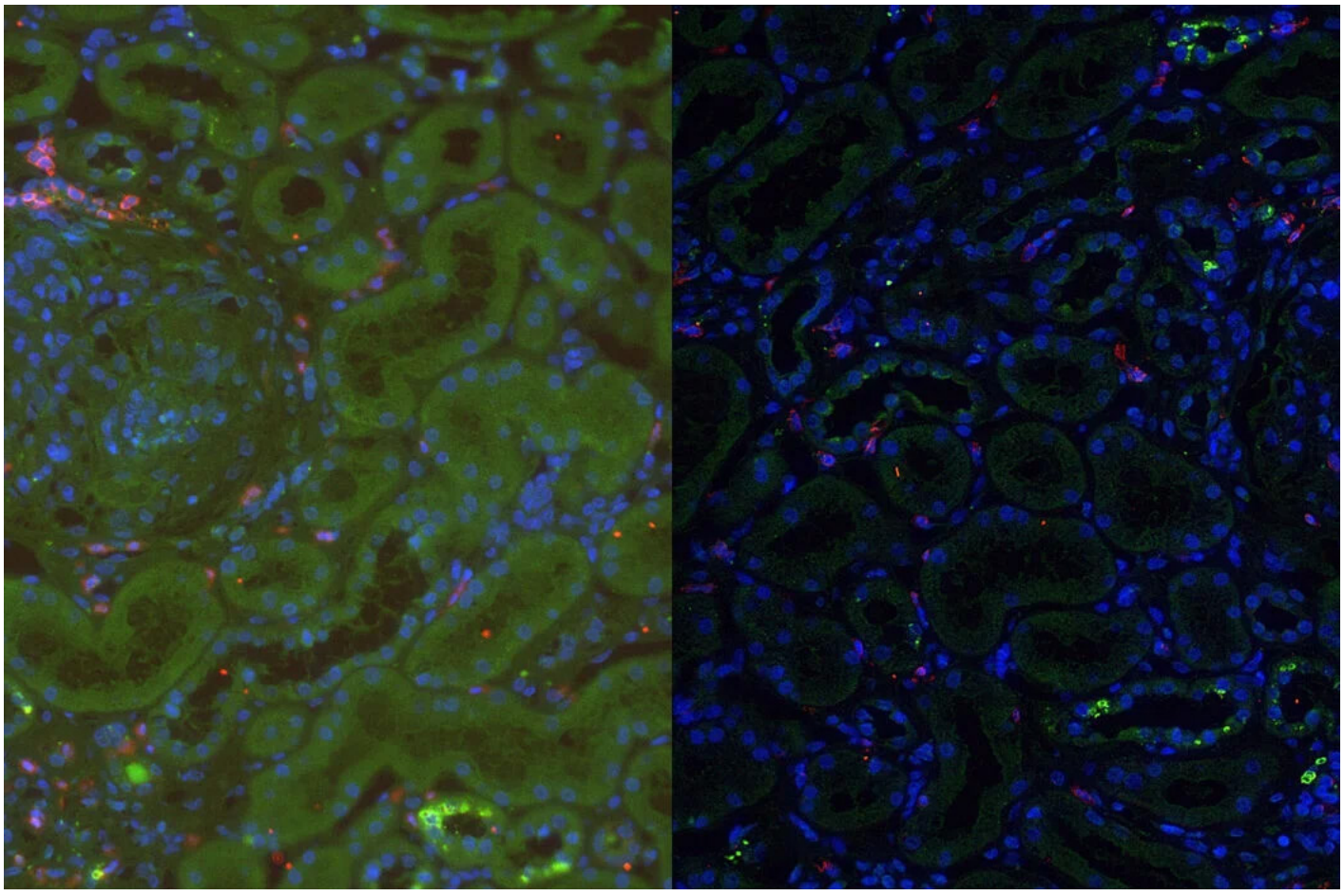
From Eye to Insight

Leica MICROSYSTEMS

STUDYING AUTOIMMUNE DISEASE

Hyperactive T lymphocytes Systemic Lupus Erythematosus (SLE)



Authors

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Abstract

This article discusses how autoimmune diseases, like systemic lupus erythematosus (SLE), can be studied more efficiently using thick, 3D kidney tissue specimens visualized with a THUNDER Imager. SLE can lead to lupus nephritis (LN) when blood vessels in the kidney become inflamed. If left untreated, eventually kidney failure could be the result. A better understanding of the initiation of LN is important for the developing treatments. This goal necessitates the study of 3D kidney specimens. To study kidney tissue efficiently, a fast screening of specimens going from overview to fine detail is a big advantage. Widefield, camera-based microscopes are easy to use and fast, but imaging 3D specimens can lead to images with reduced contrast due to an out-of-focus blur or "haze". This problem of haze is eliminated with THUNDER Imagers.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease which affects about 1.5 million people in the USA. It results in chronic inflammation of various organs leading to permanent organ damage. When SLE infects the glomeruli of the kidney, termed lupus nephritis (LN), the small blood vessels in the kidney become inflamed resulting in potential kidney failure. Understanding the disease forming pathway in LN will be important for the development of effective therapies to treat this autoimmune disease [1,2]. It has been shown that human T lymphocytes with a characteristic defect in potassium channel behavior contributes to the hyperactivity of SLE T lymphocytes [2]. Therefore, it may be possible to target ion channels for new therapies to suppress hyperactive T lymphocytes of SLE patients with a focus on their chemotactic and cytotoxic capabilities [1,2].

Challenges

For the study of kidney specimens, an imaging solution is needed for fast screening of large specimen overviews at high magnification. Widefield, camera-based fluorescence microscopy offers ease of use, speed, and detection sensitivity, however, when imaging thick 3D specimens image contrast is reduced and structural details are obscured due to the out-of-focus blur (haze) [3]. This haze is produced by detected fluorescence signals which are emitted from out-of-focus planes in the specimen [3].

Methods

Formalin-fixed paraffin-embedded (FFPE) kidney tissue biopsy specimens of LN patients were utilized for this study. Before imaging, the FFPE specimens were deparaffinized and stained with Alexa-Fluor-647-conjugated rabbit anti-human CD8 antibodies (green) and Alexa-Fluor-594-conjugated mouse anti-CD45RO antibodies (magenta) to label memory T lymphocytes, a type of white blood cell [4]. Nuclei in the specimens were stained with DAPI (blue). The specimens were imaged on the THUNDER Imager 3D Cell Culture using large volume Computational Clearing (LVCC) [3].

Results

Kidney specimens were examined for memory T lymphocyte infiltration. The images in figure 1 show the extent of T cell infiltration where magenta is detected. The THUNDER images after LVCC processing are clearer and sharper than the raw widefield images. These results show that THUNDER Imagers can reveal biological events that are typically obscured in the "haze" when imaged using conventional widefield microscopes.

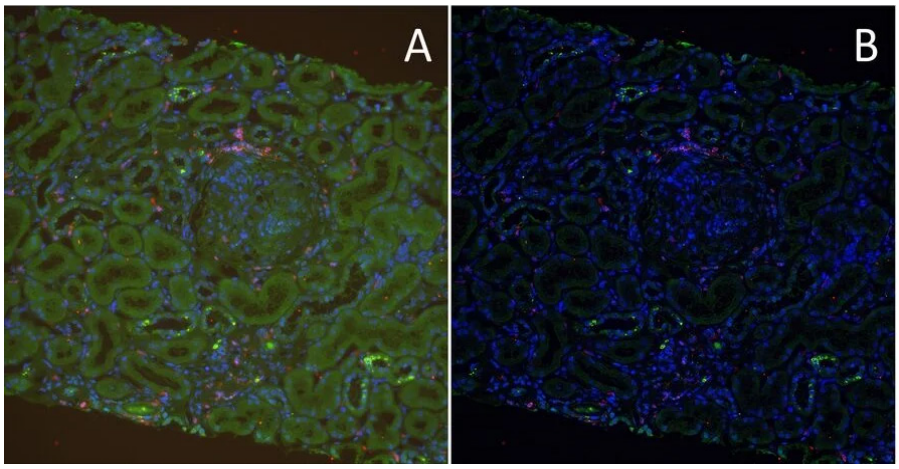

Fig. 1:An FFPE kidney biopsy specimen from a LN patient examined for memory T lymphocyte infiltration. A) Raw widefield, extended-depth-of-field image without Computational Clearing. B) THUNDER image processed with LVCC.

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