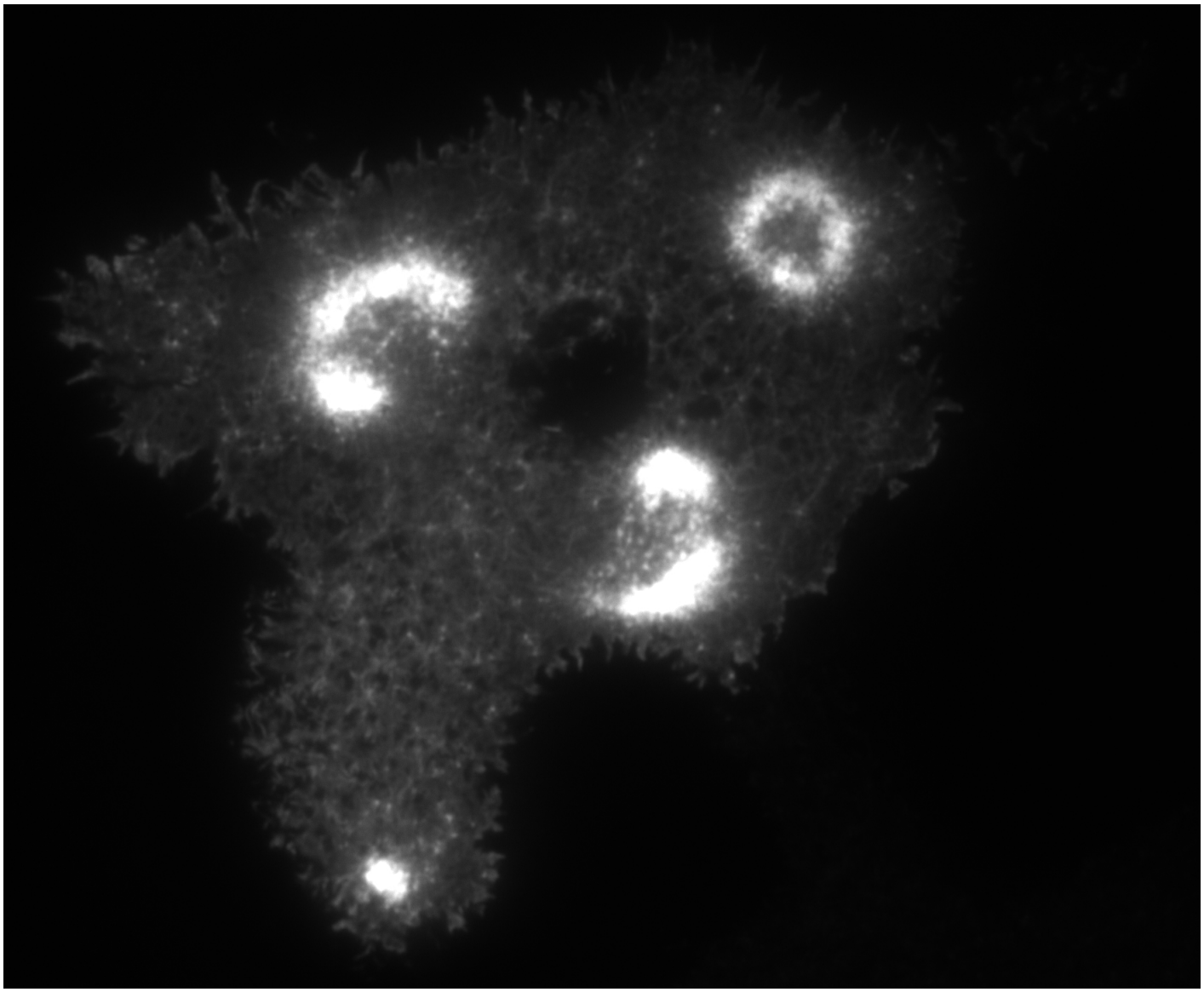
From Eye to Insight

Leica MICROSYSTEMS

STUDYING ACTIN CYTOSKELETON AND MICROFILAMENT ASSEMBLY

Superior Resolution actin imaging with TIRF



Authors

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Abstract

This article demonstrates how actin microfilament assemblies can be clearly visualized with superior resolution at the plasma membrane of a cell using a DMi8 S Infinity TIRF High Power imaging solution. To better understand how pathogens disrupt host cell signaling and cytoskeletal membrane trafficking during invasion and intracellular replication, infection of the S. frugiperda Sf21 moth larva by the baculovirus A. californica Multiple Nucleopolyhedrovirus (AcMNPV) was used as a model. The goal of the study was not only to gain insights into the interaction between the AcMNPV virus and actin, but also normal cytoskeleton functions.

Introduction

A variety of bacterial and viral pathogens target the actin cytoskeleton of host cells during infection. To gain insights into the strategies used by such pathogens to target host cell signaling, cytoskeletal membrane trafficking, and gene regulatory proteins in order to promote invasion, the insect baculovirus Autographa californica Multiple Nucleopolyhedrovirus (AcMNPV) was studied [1]. By examining the interaction between the AcMNPV virus and actin, the goal is to better understand critical mechanisms of pathogenesis as well as the normal cytoskeleton functions in uninfected cells. The main questions to answer are how actin microfilament assembly is initiated and regulated and how it functions during processes like whole cell migration, membrane trafficking, and viral pathogenesis and invasion.

The baculovirus AcMNPV is a large DNA virus that infects arthropods, in particular lepidopteran larvae (caterpillars), and is commonly used in the baculovirus protein expression system [1]. AcMNPV has a unique ability to hijack the host cell's actin cytoskeleton and utilize it for many purposes, including cell-to-cell spread and nucleocapsid assembly. Recent findings suggest that AcMNPV may also utilize the actin cytoskeleton to bypass basement membranes and ensure systemic host infection [1].

Challenges

For the study of actin microfilaments in cells, a fluorescence imaging solution is needed that allows the actin at the plasma membrane of the cell specimen to be easily resolved. Conventional widefield fluorescence microscopy is unable to achieve this resolution due to out-of-focus fluorescence signals.

Methods

Actin microfilament assembly was studied using cells of the caterpillar larva of the fall armyworm, the Spodoptera frugiperda Sf21 moth. The cells were transfected with a plasmid expressing green-fluorescent-protein-tagged action (GFP-actin), infected with AcMNPV, and then imaged 4 hours post-infection. Imaging was done at the plasma membrane/coverslip interface with superior resolution and contrast using a DMi8 S Infinity TIRF HP (High Power) imaging solution. The DMi8 S widefield microscope was equipped with a 100X/1.47 NA TIRF oil immersion objective, 488 nm excitation laser, and Hamamatsu Flash V.4.0 sCMOS camera. First, the LAS X Navigator software was used to scan the entire specimen in the petri dish and quickly identify infected cells for high-resolution imaging. The Infinity TIRF HP system, with its auto alignment feature, allows users to quickly switch between the TIRF (Total Internal Reflection Fluorescence) [2], HILO (Highly Inclined and Laminated Optical) mode [3], and EPI (Epifluorescence) [4] illumination modes.

Results

The images in figure 1 below show stills taken from two time-lapse series of the same Sf21 cells. Figure 1A shows an epifluorescence (widefield) image of the moth cells and figure 1B shows a TIRF image. The GFP-actin forms bright ring-like structures in AcMNPV-infected cells and their dynamics at the plasma membrane were more clearly observed with TIRF than epi-fluorescence imaging.

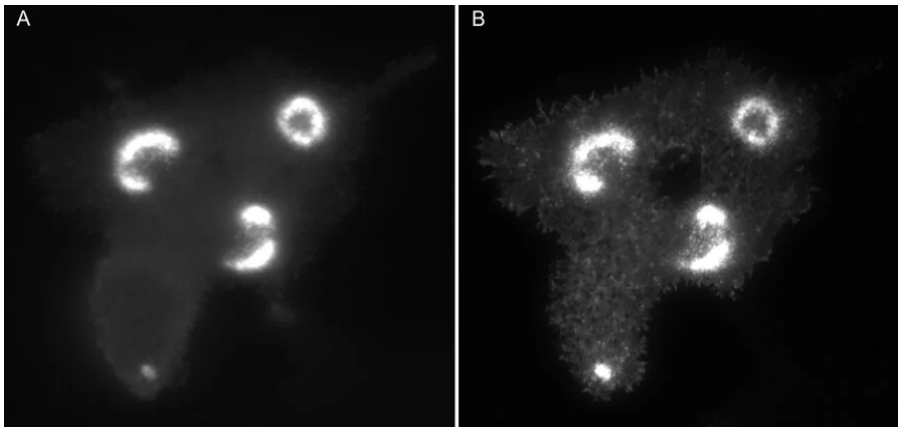

Fig. 1: Images from 2 time-lapse series of the same Sf21 moth larval cells illuminated with: A) EPI mode and B) TIRF mode. Images were acquired with the DMi8 S Infinity TIRF HP system. Courtesy of Domokos Lauko, Welch Lab, Molecular and Cell Biology, University of California, Berkeley, USA.

References

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