Sapphire FL Imaging of Multiplex Fluorescent Immunohistochemistry Slides

Introduction

Immunohistochemistry (IHC) is a widely used technique used to identify antigens in tissue specimens on slides. IHC involves using a fresh frozen or formalin-fixed paraffin-embedded (FFPE) tissue sample, or a cell block consisting of a cell suspension embedded in a paraffin block. The sample or block is thinly sliced and placed on a slide where detection of specific antigens is achieved using labeled antibodies. Slides are usually visualized using a microscope, but fluorescently labeled antibodies can also be visualized using other fluorescent imaging technology, such as a biomolecular scanner.

A value of IHC over other immunodetection techniques is that antigen expression is detected within the context of tissue morphology. The antigen's location in a cell or tissue can provide information about its role in biological processes, as well as about the integrity or developmental stage of tissues that cannot be gained from other experimental approaches. Because of this, IHC is of great value for basic research and an important tool for disease diagnosis and staging of cancer.

Traditionally, IHC experiments have used antibodies labeled with horseradish peroxidase or alkaline phosphatase with detection relying on a chromogenic substrate. With chromogenic detection, only a single antigen can be detected on a slide. However, fluorescently labeled antibodies allow for multiplex detection, greatly increasing the information that can be obtained from one slide. Multiplex fluorescent detection greatly improves diagnostic accuracy.[1]

While IHC slides are typically imaged using a microscope, fluorescent scanners like the Sapphire FL Biomolecular Imager are useful for rapid triage and whole-slide documentation. Exclusively imaging slides on a microscope can be a time consuming and cumbersome step in the experimental workflow. Using a fluorescence scanner for rapid triage allows researchers to quickly select the best slides for microscopic analysis. In addition, images of whole slides (compared to small sections of slides captured by microscopes) can supplement microscope images and assist in documentation.

This application note demonstrates the use of the Sapphire FL Biomolecular Imager to image a multiplex fluorescent immunohistochemistry experiment. Using Standard Optical Modules, the Sapphire FL is used to detect two antigens in a tissue section of mouse lung.

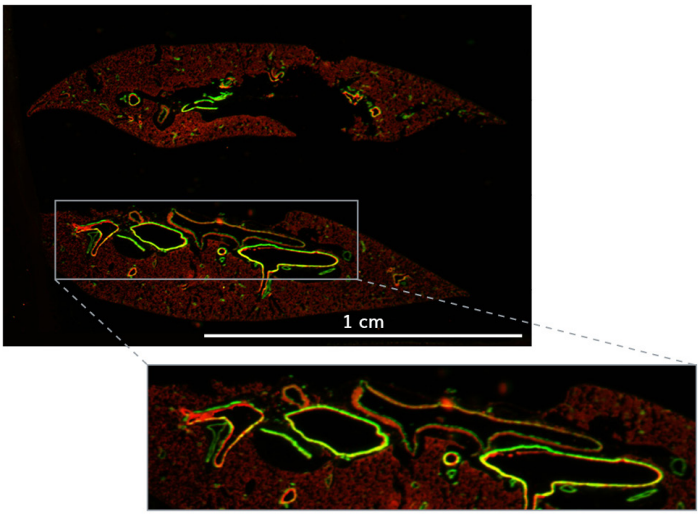

Figure 1. Mouse lung tissue slide probed for vascular endothelial (VE)-cadherin (AzureSpectra 550 nm secondary antibodie) and smooth muscle actin (SMA) (AzureSpectra 650 nm secondary antibodies). Imaged on the Sapphire FL using the 532 and 638 Standard Optical Modules (red and green, respectively) at 5µm.

Methods

Preparation of mouse lung tissue

Paraffin blocks containing mouse lung tissue were sectioned onto slides and the slides baked for 45 minutes in a 60-degree oven. The tissue sections were deparaffinized and rehydrated through a series of xylene and ethanol washes. The following washes were carried out twice for 5 minutes each: xylene, 100% ethanol, 95% ethanol, 70% ethanol, and PBS.

Antigen retrieval

Following deparaffinization and rehydration, slides were incubated in Tris-EDTA Tween® buffer (pH 9) in a rice cooker for 22 minutes. The slides were then allowed to return to room temperature for 20 minutes and washed in PBS two times for 10 minutes each wash.

Immunodetection

The sections were probed for vascular endothelial (VE) cadherin and for smooth muscle actin (SMA). A polyclonal rabbit anti-VE cadherin (Abcam product #ab33168) primary antibody and a monoclonal mouse anti-SMA (Dako product #M0851) primary antibody were used. Tissue sections were blocked for 12 minutes in Power Block™ (BioGenex product #HK085-5K). The primary antibodies were diluted in PBS buffer containing 0.5% Triton™; the rabbit anti-VE cadherin antibody was diluted 1:100 and the mouse anti-SMA antibody was diluted 1:200. Slides were incubated with primary antibodies overnight in a cold room. Unbound primary antibody was removed with a series of washes: TBS for two washes of 5 minutes each followed by one wash in TBS-Tween®.

Primary antibodies were detected using fluorescently labeled secondary antibodies; AzureSpecta goat-anti-rabbit 550 (Azure Biosystems product #AC2158) and AzureSpectra goat-anti-mouse 650 (Azure Biosystems product #AC2166). The secondary antibodies were diluted 1:500 in PBS buffer containing 0.5% Triton™. Slides were incubated in the secondary antibody solution for one hour at room temperature. Unbound secondary antibodies were removed with a series of washes: TBS for two washes of 5 minutes each followed by one wash in TBS-Tween®. The slides were allowed to dry, then coverslipped and imaged.

Imaging

Slides were imaged on a Sapphire FL using the 532 and 638 Standard Optical Modules and the Extended Dynamic Range (EDR) function. The focus type was Slide, which sets the focus +1.00 mm above the glass imaging surface. Images were captured at 5-micron resolution with the quality setting set to High.

Results and Discussion

Figure 1 depicts an image of a multiplex fluorescent IHC experiment captured on the Sapphire FL Biomolecular Imager. The slide in Figure 1 contains sections of mouse lung tissue probed for VE-cadherin and SMA and detected using two fluorescently labeled secondary antibodies. VE-cadherin appears red in the image and was detected using the Sapphire FL 532 Standard Optical Module. SMA appears green in the image and was detected using the Sapphire FL 638 Standard Optical Module. Tissue morphology is easily seen in the 5-micron image. In Figure 1, a region of lung tissue is enlarged, allowing the localized SMA staining to be visualized in greater detail.

The Sapphire FL Biomolecular Imager is a powerful system for multiplex fluorescent imaging of a wide range of sample types. With its software-controlled adjustable focus, the Sapphire FL can capture clear images of tissue sections on slides (as demonstrated in this application note), as well as flat samples (like Western blots), and samples with depth (like multi-well plates or small model organisms). To facilitate IHC and other slide imaging experiments, the Sapphire FL has an available slide holder accessory.

Scientific investigation is never limited by the Sapphire FL's imaging technology. The Sapphire FL is a modular system, providing ultimate flexibility for fluorescence imaging experiments. Multiple Standard Optical Modules are available for imaging commonly used dyes. The excitation laser modules and emission filter modules can be interchanged to best match any desired fluorophore. Custom Optical Modules are also available by request.

In addition to multiplex fluorescence, the Sapphire FL carries out white light, UV, and near-infrared fluorescence imaging, as well as chemiluminescence and phosphor imaging. Learn more about the Sapphire FL at the Azure Biosystems website.

References

1. Sheng W, Zhang C, Mohiuddin TM, et al. Multiplex immunofluorescence: a powerful tool in cancer immunotherapy. Int J Mol Sci. 2023;24(4):3086

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