Fluorescent IHC Imaging of Tumor Tissue Arrays on the Sapphire FL

Introduction

Tissue microarrays are a widely used, high-throughput technique for the analysis of tissue specimens. First described in 1998, tissue microarrays are histological slides containing as many as 1000 unique samples.[1] Tissue microarrays are created by inserting tissue cores into a prepared paraffin block in a grid arrangement. The block can be sliced perpendicular to the cores to generate slides containing cross-sections of the tissue cores. More than 200 slices can be obtained from a single block, allowing hundreds of experiments to be carried out on the same samples.

Many types of samples can be used to create tissue arrays including frozen tissue, formalin-fixed, paraffin-embedded (FFPE) tissue, and cell blocks.[2] Today, many tissue microarrays are commercially available and are particularly valuable for studying tumors and cancer biology.

In addition to facilitating high-throughput experiments, advantages of tissue microarrays include standardized processing and imaging conditions and conservation of time and materials. All samples on the slide are processed in parallel so are subject to identical experimental and imaging conditions, reducing variability. Tissue arrays help conserve rare clinical specimens, maximizing the number of experiments that can be conducted on a single tissue sample. They also conserve reagents and time compared to processing hundreds of slides and tissue sections separately.

Tissue microarrays offer a high-throughput approach to histology, immunohistochemistry (IHC), fluorescence in situ hybridization (FISH), and other experiments carried out on tissue sections. They facilitate comparisons among different sample types, such as comparing tumor to normal tissue, comparing different types of tissue or different type of tumors, or comparing different tumor stages. Tissue microarrays have a wide range of applications such as validating biomarkers and identifying or confirming differences in gene or protein expression between samples.

Tissue microarrays are typically imaged under a microscope, one sample at a time. In this application note, we describe using the Sapphire FL Biomolecular Imager to capture images of the entire array. These whole-slide images can be used to catalog slides and provide an initial survey of staining success before proceeding with higher-resolution imaging. In this note, tissue microarrays stained with fluorescent stains or probed with antibodies in a two-color IHC experiment are imaged on the Sapphire FL.

Materials and Methods

Multiple Tumor Tissue Arrays (BioChain, catalog # T8235714d-5) were stained and probed. The arrays contain a variety of tumor types as well as paired samples of tumor and normal tissue. The layout of the array is shown in Table 1.

Before staining or probing, the arrays were deparaffinized and rehydrated by immersing the slides in the following series of solutions:

- Xylene, 3 washes, 5 min each
- 100% ethanol, 2 washes, 10 minutes each
- 95% ethanol, 2 washes, 10 minutes each
- 70% ethanol, 2 washes, 10 minutes each
- 50% ethanol, 2 washes, 10 minutes each
- Deionized water, 2 washes, 5 minutes each

The slides were then heated to 60 °C for 30 minutes and cooled to room temperature before incubating in permeabilization buffer (1% FBS, 0.4% Triton X-100 in PBS) two times for 10 minutes each incubation.

Normal placenta (+ control)	Paraffin (- control)	Ovary Adenocar-cinoma, Mucous	Ovary Adenocar-cinoma	Ovary Adenocar-cinoma	Ovary Cystadeno-carcinoma, Serous	Ovary Adenocar-cinoma	Ovary Serous Papillary Cystad-enoma	Matched normal tissue	Ovary Adenocar-cinoma	Matched normal tissue
Bladder Transitional Cell Carcinoma	Bladder Transitional Cell Carcinoma	Bladder Transitional Cell Carcinoma	Bladder Transitional Cell Carcinoma, Papillary	Bladder Transitional Cell Carcinoma	Bladder Transitional Cell Carcinoma, Papillary	Bladder Transitional Cell Carcinoma	Bladder Transitional Cell Carcinoma	Matched normal tissue	Bladder Transitional Cell Carcinoma, Papillary	Matched normal tissue
Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Breast Invasive Ductal Carcinoma	Matched normal tissue	Breast Invasive Ductal Carcinoma	Matched normal tissue
Kidney Clear Cell Carcinoma	Kidney Clear Cell Carcinoma	Kidney Clear Cell Carcinoma	Kidney Clear Cell Carcinoma	Kidney Clear Cell Carcinoma	Kidney Clear Cell Carcinoma	Kidney Clear Cell Carcinoma	Kidney Transitional Cell Carcinoma	Matched normal tissue	Kidney Clear Cell Carcinoma	Matched normal tissue
Uterus Endometrial Adenocar-cinoma	Uterus Endometrial Adenocar-cinoma	Uterus Endometrial Adenocar-cinoma	Uterus Endo-metrioid Carcinoma	Uterus Squamous Cell Carcinoma	Uterus Squamous Cell Carcinoma	Uterus Adenocar-cinoma	Uterus Endometrial Adenocar-cinoma	Matched normal tissue	Uterus Endometrial Adenocar-cinoma	Matched normal tissue
Lung Squamous Cell Carcinoma	Lung Squamous Cell Carcinoma	Lung Squamous Cell Carcinoma	Lung Squamous Cell Carcinoma	Lung Squamous Cell Carcinoma	Lung Squamous Cell Carcinoma	Lung Adenocar-cinoma	Lung Squamous Cell Carcinoma	Matched normal tissue	Lung Squamous Cell Carcinoma	Matched normal tissue

Table 1. Tumor tissue microarray sample layout

Fluorescent tissue staining

To demonstrate imaging of a fluorescently stained tissue array, the array was probed with AlexaFluor™ 488-labeled phalloidin, a high-affinity probe for filamentous actin (F-actin), and with Hoechst stain, which binds double-stranded DNA to visualize nuclei.

Staining was conducted by incubating the slide in a solution containing Hoechst stain (10 µg/ml) and 1X AlexaFluor™ 488-Phallodin in Azure Fluorescent Blot Blocking Buffer (Azure Biosystems, catalog #AC2190) for 30 minutes at 60 °C. The slide was then washed in Azure Blot Washing Buffer (Azure Biosystems, catalog #AC2113) 3 times for 5 minutes each wash.

The slide was imaged on the Sapphire FL at 10 µm resolution. Hoechst stain was detected using the 375 Standard Optical Module with intensity set to 5. AlexaFluor™ 488 Phalloidin was detected using the 488 Standard Optical Module with intensity set to 8. The slide was placed in the slide holder accessory and the Slide (+1.00 mm) focus setting was used. Imaging quality was set to Highest. Note that when the slide holder accessory is used, the slide should be placed upside down such that the sample is towards the glass, and the “Slide” focus setting should be used.

Immunohistochemistry

To demonstrate imaging of immunohistochemistry microarrays, an array was probed with antibodies directed against Her2 and p53 and detected using fluorescent secondary antibodies.

First, any nonspecific binding sites were blocked by incubating the slide in Azure Fluorescent Blot Blocking Buffer for 30 minutes at room temperature.

The slide was then hybridized with a primary antibody solution consisting of 1:100 dilutions of each primary antibody in Azure Fluorescent Blot Blocking Buffer. The antibodies used were mouse anti-P53 (CST catalog #48818S) and rabbit anti-HER2 (CST catalog #2165S). The antibody solution was pipetted into the slide chamber, the chamber sealed, and the antibodies allowed to hybridize overnight at 4 °C. Unbound antibody was removed by washing the slide in Azure Blot Washing Buffer three times for 5 minutes each.

The slide was then incubated for 1 hour at room temperature in a secondary antibody solution consisting of two secondary antibodies diluted 1:5000 in Azure Fluorescent Blot Blocking Buffer. The secondary antibodies used were donkey anti-rabbit IR700 (Advansta R-05785-250) and donkey anti-mouse 800 (Advansta R-05784-250).

The slide was then washed in 1X Azure Fluorescent Blot Washing Buffer (Azure Biosystems, catalog #AC2145) three times for 5 minutes each and then in 1X PBS one time for 5 minutes. The slide was then dried and imaged.

The slide was imaged on the Sapphire FL. HER2 was detected using the 685 Standard Optical Module with intensity set to 10. P53 was detected using the 784 Standard Optical Module with intensity set to 10. The resolution was set to 10 µm. The slide was placed in the slide holder accessory and the Slide (+1.00 mm) focus setting was used. Imaging quality was set to Highest.

Results

Figure 1 shows a tumor tissue array stained for DNA (nuclei) and actin, captured on the Sapphire FL. In Figure 1A, the images captured in the two fluorescent channels are overlaid; DNA appears blue, and actin appears green. Figures 1B and 1C show the single-channel images. Differences between tumor and tissue types in the relative amount of staining by each dye are apparent.

Figure 2 depicts a tissue array probed with antibodies for P53 and HER2, two proteins important to tumorigenesis. In Figure 2A, the images captured in each fluorescent channel are overlaid; HER2 appears red and P53 appears green. Differential staining between tissue and tumor types is apparent. In addition, some tissue morphology can be visualized in this 10 µm image. Figures 2B and 2C show zoomed-in views of well A10 in each single-channel image. For finer analysis, the slide should be imaged using a microscope.

The Sapphire FL is an excellent choice for scanning tumor tissue arrays stained with fluorescent dyes or probed with fluorescent antibodies. The Sapphire FL can capture images with resolution as high as 5 µm, useful for cataloguing and categorizing slides, visualizing global differences in staining intensity between samples, and providing some histological detail within the tissue samples. Slides of interest can be imaged under a

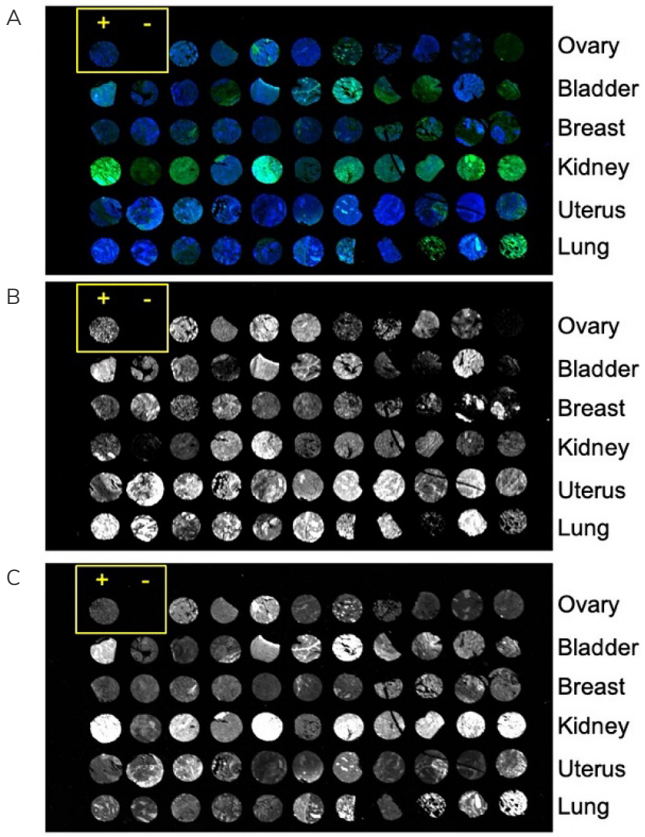

Figure 1. A. Overlay of two-channel fluorescent image of a tumor tissue microarray stained to detect F-actin (green) and DNA (blue). The top left corner contains positive and negative controls. B. Single-channel image from A captured using the 375 Standard Optical Module to detect Hoechst stain. C. Single-channel image from A captured using the 488 Standard Optical Module to detect F-actin.

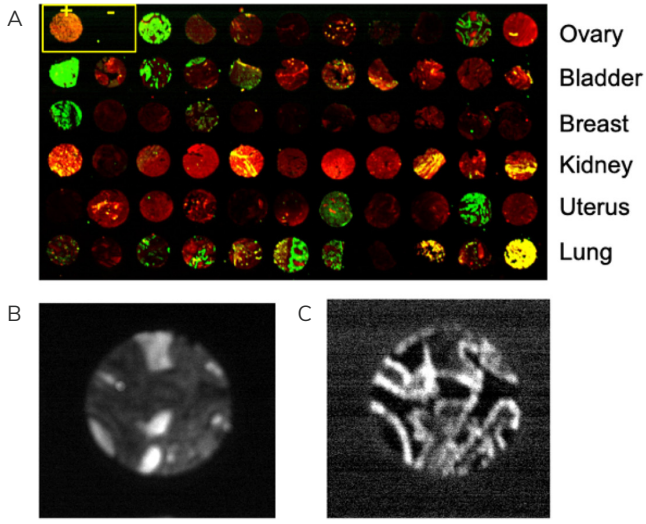

Figure 2. A. Overlay of two-channel fluorescent imaging of a tumor tissue microarray probed with antibodies to detect HER2 (red) and P53 (green). The top left corner contains positive and negative controls. B. Single-channel image of well A10 captured in the 685 Standard Optical Module to detect HER2. C. Single-channel image of the well A10 captured in the 784 Standard Optical Module to detect P53.

microscope for higher resolution imaging and analysis of subcellular stain location.

An advantage of the Sapphire FL for tissue array scanning is the ability to conduct up to four-color fluorescent imaging. The experiments presented here demonstrate two-color visible fluorescence and two-color near-IR fluorescence. The AzureSpot Pro analysis software makes it easy to delineate and measure the fluorescent signal of regularly arrayed samples such as those on a tissue microarray, protein array, or microtiter plate. Automated analysis facilitates comparing relative fluorescent intensities among samples.

In conclusion, the Sapphire FL is a great option for scanning fluorescently labeled tumor tissue microarrays. Its high-resolution, multi-color fluorescent capacity, and user-friendly software make the Sapphire FL a powerful system for tissue array imaging and analysis.

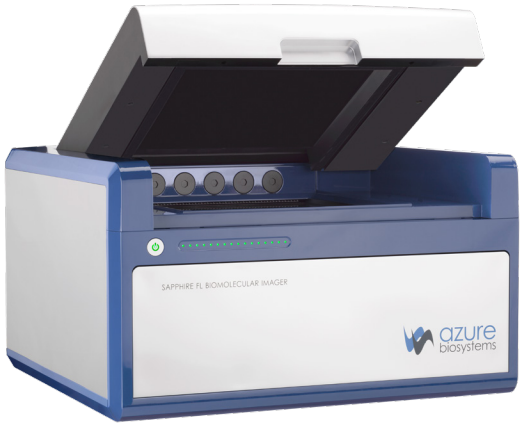

Figure 3. Sapphire FL is a laser scanner designed for multicolor fluorescent imaging, NIR fluorescent imaging, chemiluminescence, phosphor imaging, and more.

References

1. Kononen J, Bubendorf L, Kallioniemi A, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med.* 1998;4(7):844-847.
2. Koo M, Squires JM, Ying D, Huang J. Making a Tissue Microarray. *Methods Mol Biol.* 2019;1897:313-323.

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MM-0145 R3