Imaging Thick-Sectioned Slides with the Sapphire FL Biomolecular Imager

Introduction

Histologic analysis serves as an important tool in biology and medicine. It is used in the characterization and diagnosis of disease, and to classify the progression of cancer and responses to treatment. Typically, thin sections of tissue are examined under a microscope after being stained to better differentiate structures or to identify specific molecules or cell types. For examination under a light microscope, sections are usually between 5 and 20 micrometers thick.

Thicker tissue sections can provide more information about structure and connectivity within a tissue. However, as section thickness increases, the tissue becomes more difficult to image because the tissue scatters light. Optical clearing agents can be used to try to minimize cloudiness resulting from light scatter. These chemicals perfuse the tissue and match the refractive index of the tissue.[1]

FluoTissue® slides (SUNJin Lab) consist of pre-stained samples of different mouse tissues. The sections on these slides are 550 micrometers thick, about 100 times thicker than typical tissue sections. The sections are cleared and mounted using an optical clearing agent to minimize artifacts. These slides are designed for demonstration and training of high magnification imaging and 3D reconstruction using confocal microscopy.

This application note demonstrates imaging FluoTissue® slides using the Sapphire FL Biomolecular Imager. Capturing a lower-magnification image of an entire slide or tissue section can be valuable to document overall staining, identify representative regions for closer examination, and visualize regions of interest in context. A method to identify the optimal laser intensity and focal plane for imaging these thick, fluorescently labeled tissue sections is presented.

Materials and Methods

Four slides containing fluorescently labeled, thick sections of mouse tissue samples were imaged on the Sapphire FL. The slides (FluoTissue® slides, SUNJin Lab) included mouse kidney (Catalog #PS001), mouse intestine (Catalog #PS002), mouse brain (Catalog #PS003), and mouse liver (Catalog #PS004). Tissue sections were 550 microns thick and were fixed, cleared, and mounted in RapiClear® 1.52 reagent (Catalog #RC152001). The tissue sections were pre-stained by the manufacturer with fluorescent labels according to the table below (Table 1).

Fluorescent Labels	Sapphire FL Channel	Display Color in Images	Target			
			Kidney	Intestine	Brain	Liver
Alexa Fluor® 488	488 Standard Optical Module	Green	Blood vessels	Blood vessels	Blood vessels	Blood vessels
SYTOX® Orange	532 Standard Optical Module	Blue	Nuclei	Nuclei	Nuclei	Nuclei
Alexa Fluor® 647	638 Standard Optical Module	Red	Peripheral nervous system	Lymphatic vessels	Dopaminergic neurons	Peripheral nervous system

Table 1. Fluorescent stains and targets used on each FluoTissue® slide.

Imaging

Setup

Perhaps due in part to the greater penetration of light into optically cleared samples which can increase fluorescence,[2] the fluorescence signal from these slides was very strong. An optional 1.0 OD Absorptive Neutral Density (ND) Filter (Product #48-090, Edmund Optics) was placed between the slide and the glass imaging surface to reduce the amount of transmitted light by 90%. The slide was placed coverslip down (facing the glass), slightly above the optional ND filter. The optional ND filter and the slide were suspended using custom apparatuses to eliminate Newton's rings interference artifacts caused by glass-on-glass contact.

Selection of optimal focal plane and laser intensity

For each slide, a SmartScan was performed at an estimated height setting to determine a starting intensity level for each laser. To determine the optimal focal plane, a Z-stack of images was collected at 25-micron, High Quality resolution, covering 12 focal heights, with a focus delta of 0.20 mm. For this step, laser intensity settings were chosen a few levels lower than the level chosen by the initial SmartScan to minimize the chances of photo-bleaching that can occur with multiple long, high-resolution scans. The images were reviewed to determine a tight range of focal planes that provided the sharpest images.

To fine-tune the optimal focal plane, a second Z-stack was performed at 10-micron, Highest Quality resolution, covering 6 focal heights, with a focus delta of 0.10 mm using the same intensity settings used for the lower resolution Z-stack. The images were reviewed to determine the optimal focal plane, considering a combination of which plane looked the sharpest by eye with which plane gave the strongest signal from the region of interest.

Once the optimal focal plane was determined, a SmartScan (or EDR scan if more appropriate; samples with a wide dynamic range are better-suited to EDR scans) was carried out at 10-micron resolution using the Highest Quality setting.

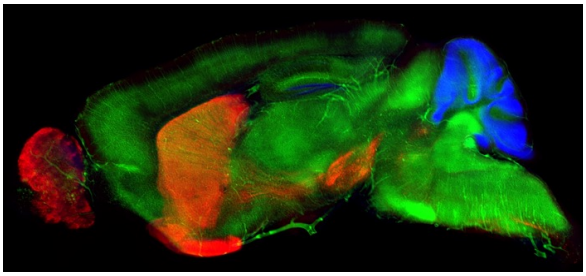
The slides were imaged in three channels on the Sapphire FL as described in Table 1.

The focus and laser intensity settings used for each dye on each slide are shown in Table 2.

Tissue	Focus	Resolution	Quality	Scan type	Laser Intensity		
					488	532	638
Brain	+3.65	10 micron	Highest	SmartScan	10	L4	4
Intestine	+3.75	10 micron	Highest	SmartScan	10	3	5
Kidney	+3.85	10 micron	Highest	SmartScan	10	4	7
Liver	+3.80	10 micron	Highest	EDR			

Table 2. Focal plane and laser intensity settings used in the final imaging of each slide

A



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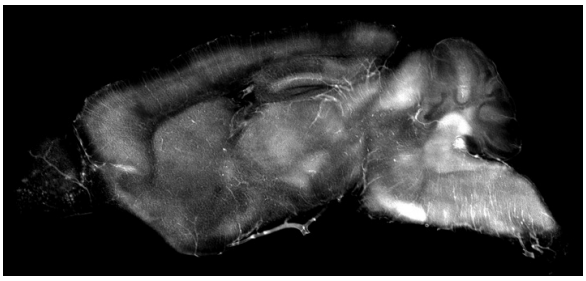

C

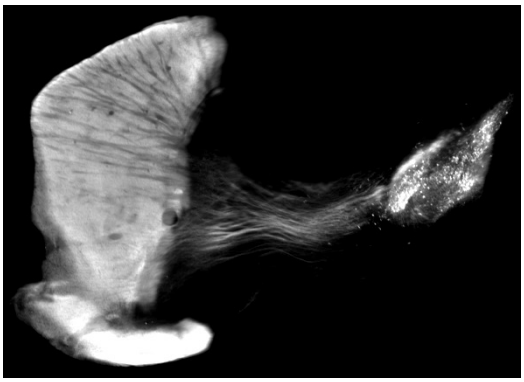

Figure 1. Mouse brain imaged on the Sapphire FL. A. Whole slide, three color image of tissue stained for blood vessels (Alexa Fluor® 488, green), nuclei (SYTOX® Orange, blue), and dopaminergic neurons (Alexa Fluor® 647, red). B. Whole slide, showing the 488 channel only (Alexa Fluor® 488, blood vessels). C. Zoomed view of the 638 channel showing dopaminergic neurons (Alexa Fluor® 647). Images are captured at 10 micron resolution.

Results

The Sapphire FL captured 10-micron, muti-color fluorescent images of very thick sections of four different mouse tissues. By selecting the optimal focal plane and laser intensities as described in the methods section, the Sapphire FL captured clear images revealing details of tissue structure using sections 100-fold thicker than typical tissue sections.

A three-color image of mouse brain (Figure 1A) shows distinct brain regions enriched in blood vessels (shown in green), nuclei (shown in blue), and dopaminergic neurons (shown in red). The image of each channel can be viewed independently. The 488 nm channel shows staining for blood vessels only (Figure 1B) while a zoomed-in image of the 638 nm channel shows greater detail of regions enriched in dopaminergic neurons (Figure 1C).

The three-color image of mouse intestine (Figure 2) reveals concentric arrangement of discrete layers enriched in blood vessels (green), nuclei (blue), and lymphatic vessels (red).

Images were also captured of mouse liver (Figure 3) and kidney (Figure 4).

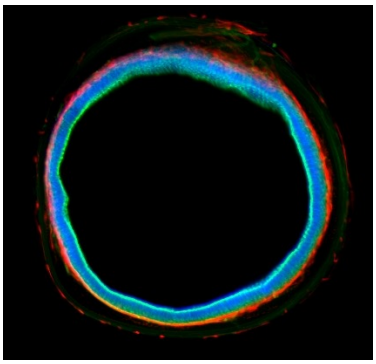

Figure 2. Mouse intestine imaged on the Sapphire FL. Three-color image of tissue stained for blood vessels (Alexa Fluor® 488, green), nuclei (SYTOX® Orange, blue), and lymphatic vessels (Alexa Fluor® 647, red). Image was captured at 10 micron resolution.

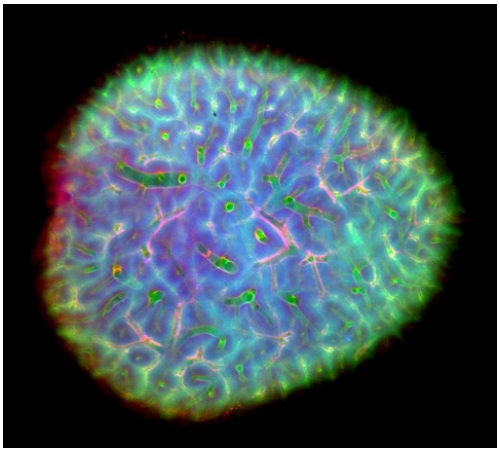

Figure 3. Mouse liver imaged on the Sapphire FL. Three-color image of tissue stained for blood vessels (Alexa Fluor® 488, green), nuclei (SYTOX® Orange, blue), and peripheral nervous system (Alexa Fluor® 647, red). Image was captured at 10 micron resolution.

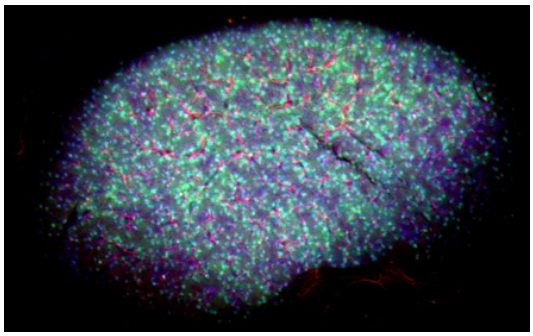

Figure 4. Mouse kidney imaged on the Sapphire FL. Three-color image of tissue stained for blood vessels (Alexa Fluor® 488 green), nuclei (SYTOX® Orange, 532, blue), and peripheral nervous system (Alexa Fluor® 647, red). Image was captured at 10 micron resolution.

Discussion

While histologic analysis often relies on microscopy to examine subcellular structures, capturing images of entire slides or tissue sections offers key advantages for documenting slide composition and large-scale characterization of tissue structure and staining. Such capabilities cannot be achieved using higher magnification alone. Imaging tissue slides, including FluoTissue® slides, which contain sections many times thicker than standard preparations, on the Sapphire FL Biomolecular Imager permits the capture and assessment of whole slides at high resolution.

The SmartScan and Z-stack imaging functions of the Sapphire FL facilitate the identification of the optimal focal plane and laser intensity for imaging such tissue slides. An initial SmartScan may be used to identify starting laser intensities. Thereafter, laser intensities a few steps below the intensity identified by SmartScan may be used to collect Z-stacks of images to zero in on the optimal focal plane while reducing the risk of photobleaching during high-resolution scans.

The Sapphire FL Biomolecular Imager carries out fluorescence, phosphor, and chemiluminescence imaging, for a wide variety of sample types. Learn more about the Sapphire FL at the Azure Biosystems website.

References

1. Richardson DS, Lichtman JW. Clarifying Tissue Clearing. Cell. 2015;162(2):246-257. doi: 10.1016/j.cell.2015.06.067.
2. Shariati BKB, Ansari MA, Khatami SS, Tuchin VV. Multimodal optical clearing to minimize light attenuation in biological tissues. Sci Rep. 2023;13(1):21509. doi: 10.1038/s41598-023-48876-x.

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