Application Note

Fluorescence Scanning



Whole Slide Imaging using Multiple Fluorescence Channels

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Abstract

Whole Slide Imaging is a technology that digitally scans and archives tissue slides. With applications in digital pathology as well as clinical and basic research, this method has become very popular over the last decade. The MMI CellScan system has been developed to combine whole slide imaging with the full functional flexibility of a research microscope. In this study, we demonstrate that the MMI CellScan slide scanner, with its sensitive CMOS camera and high-precision microscope stage, is capable of capturing and compiling multi-color fluorescence images.

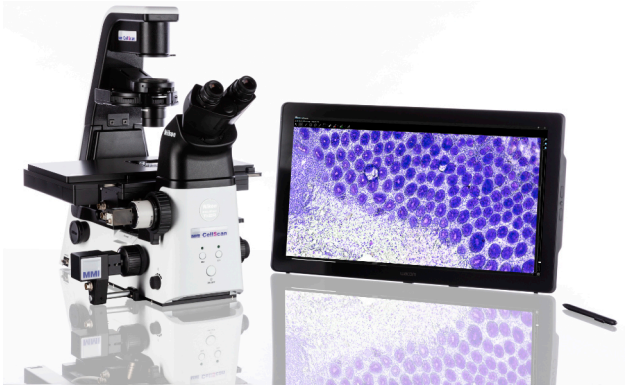


Fig. 1: MMI CellScan system mounted on the Nikon Ts2R inverted microscope. This system is compatible with a variety of microscope brands and models and can be integrated with all MMI cell isolation systems for diverse research applications.

Introduction

Whole slide imaging (WSI), also known as virtual microscopy, is a technology used to scan and digitally archive slides at high resolution. Slide scanners capture individual images of each field of view across the entire microscopy slide. These images are then stitched together to create a single high-resolution image file.

The technology of whole slide imaging has developed rapidly over the last decade, largely due to significant advancements in the storage of large datasets, barcoding, file tracing, and data exchange. Telepathology workflows have emerged, enabling pathologists to consult with experts on specific tissue types or diseases and to discuss complex cases with colleagues

worldwide using digital slides. Furthermore, digital slides are less susceptible to quality issues such as color changes, photobleaching, or degradation from long-term storage. By digitally archiving tissue slides, researchers can analyze them with the same confidence after years or even decades.

The MMI CellScan is a whole slide imaging system built on standard research microscopes. This slide scanner can utilize all the functionalities of the research microscope, making it highly versatile, flexible, and upgradeable. Scanning is not limited to a single resolution; changing objectives or switching between brightfield and fluorescence imaging modes can be done with just one mouse click (when installed on a fully motorized microscope such as the Nikon Ti2E or Olympus IX83). In addition to standard microscopy slides, the MMI CellScan is compatible with various sample formats, including well plates, dishes, and MMI Membrane Slides for laser microdissection.

Here, we present the MMI CellScan whole slide imaging system applied to fluorescent tissue sections. Digital images of individual fluorescent channels are acquired and then automatically merged to create a single multi-color image. These images are saved in the open BigTIFF format, which is compatible with our free MMI CellViewer as well as many other slide viewers and image analysis software packages.

Material and Methods

A tissue section of mouse kidney was mounted onto a standard microscope slide. The tissue was stained with DAPI to visualize nuclei and CF-594 to mark laminin. The slide was placed into the slide holder on the stage of an inverted microscope outfitted with the MMI CellScan module.

Using the 'Preview Scan' function of the MMI CellScan, the slide was first scanned at a magnification of 4x. The software automatically identified the region where tissue was present. A focus map was automatically assigned by the MMI CellScan software. The pre-defined tissue area was then scanned at a 20x magnification (CFI S Plan Fluor ELWD 20xC objective) using the pre-selected DAPI and FITC channels to view DAPI and CF-594 stains. Separate images for both channels were automatically saved as BigTIFF in a dedicated folder. The images were automatically overlaid using the free MMI CellViewer software. Individual channels can be selected and de-selected, and their intensity can be individually adjusted for optimal image analysis.

Results

The MMI CellScan is a flexible microscope-based slide scanner with various applications in clinical and basic research. Here, we demonstrate that the MMI CellScan fulfills all the needs of a standard slide scanner and can even scan full-resolution slides with various fluorescence colors to generate multi-color images.

The MMI CellTools software controls the acquisition of single-channel fluorescence images, which are then digitally merged to generate one multi-color image. The MMI CellTools software platform offers additional features and parameter settings to efficiently scan slides in one or more fluorescence imaging modes. The autofocus function, for example, automatically sets reference points in the z-dimension to optically flatten uneven samples. Thus, the resulting image is in focus at every position, which is crucial for detailed and automatic image analysis.

To assess the capabilities of the MMI CellScan, a double fluorescently stained tissue slide using DAPI and CF-594 laminin stain was scanned as a full-resolution image file. With the sensitive CMOS MMI camera and the fast microscopy stage, the MMI CellScan can scan a 15 mm x 15 mm section using the 20x objective in less than 1 minute per fluorescence channel. Of course, the scanning time will depend on the brightness of the fluorescent stain and thus on the exposure time during image acquisition. However, the ability to use a range of objective magnifications and numerical apertures means the system is not limited. The image was then automatically overlaid and saved in a pyramid BigTIFF file format.

For image analysis, the image was loaded into the free MMI CellViewer software. The binning algorithm implemented in the MMI CellViewer enables rapid loading of large image files and allows for fast and seamless zooming to full resolution, even with multiple superimposed fluorescence images. Single fluorescence channels of the composed image are accessible for individual adjustments, such as brightness or contrast (Figure 2).

As the MMI CellScan accurately saves absolute position information, the individual fluorescent images can be perfectly overlaid to create a single image with multiple colors (Figure 3). This accurate positioning can also be used to mark specific cells or areas in the scanned image for subsequent laser microdissection. This workflow minimizes exposure of the sensitive sample to UV light, thereby reducing photobleaching effects.

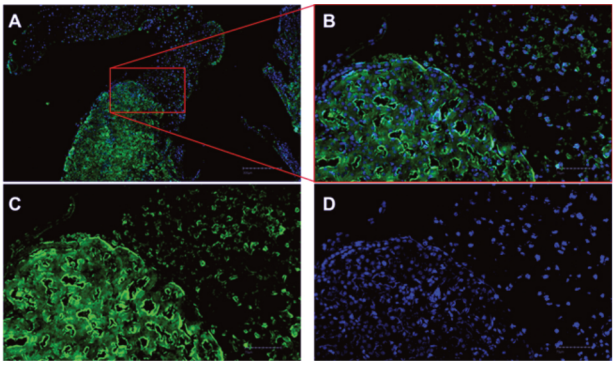


Fig. 2: Image analysis using the free MMI CellViewer. The image opens at the lowest magnification to fit the window size. Seamless and rapid zooming to full resolution is enabled by a binning algorithm. **(A)** Overview image of the tissue section at low magnification. Both channels DAPI (blue) and CF 594 (green) are shown. **(B)** Zooming to full resolution provides further details of the sample. **(C)** Same picture section as shown in (B) but now only displaying the CF-594 channel. **(D)** Same picture section as shown in (B) but now only displaying the DAPI channel.

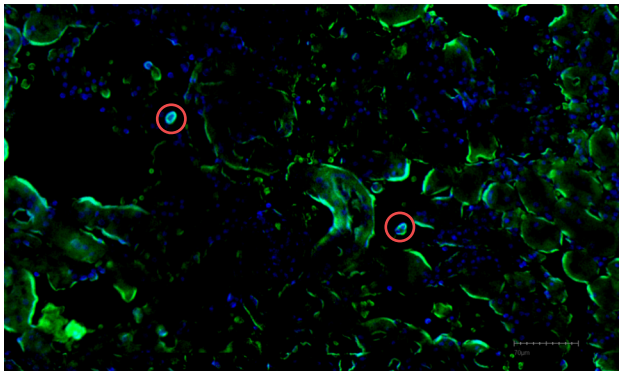


Fig. 3: Overlay image shows accurate positioning with highlighted (red circles)

Discussion

In this study, we demonstrate that the MMI CellScan is a high-performance slide scanner capable of scanning full-resolution images in several fluorescence channels. Multi-color fluorescence images are automatically composed by the integrated software, which allows for adjustment of the individual channels and saves them in an open file format.

A key benefit of acquiring a whole-sample image is the reduction of sampling bias, which can occur when only selective areas are acquired. Therefore, this approach increases the robustness of the data and the validity of the experiment.

Since the MMI CellScan is a microscope-based scanning system, it offers the full flexibility of a research microscope and can additionally be employed for both standard and fluorescence microscopy applications. Moreover, the MMI CellScan can use all objectives; therefore, it can be optimized for a range of samples, such as slides or culture dishes.

In addition to standard consumables, the MMI CellScan can also scan MMI Membrane Slides dedicated to Laser Microdissection. Interestingly, the MMI CellScan can be integrated with the MMI CellCut system to uniquely and seamlessly combine whole-slide imaging and laser microdissection. This workflow reduces light exposure of the sensitive sample during scanning and thus minimizes photobleaching effects. Furthermore, the scanned image files can be shared with colleagues and collaboration partners, including all labels and annotations. The MMI CellScan is therefore a highly versatile and flexible scanning system, providing useful features and options for optimal scanning of any sample type in both brightfield and fluorescence.

Your benefits

- Whole slide scanner with the highest flexibility: Compatible with all objectives, numerous research microscopes and all possible imaging modes
- Scan your whole slides in 5 dimensions: XY-Scan, Z-stacks, time-lapse and multifluorescence
- Seamlessly operate from any location
- High-performance whole slide scanner: Less than 60 seconds to scan a 15 x 15 mm section (20x objective)
- User-friendly operation: Simply place your slides under the microscope and activate the device

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