Application Note MMI-CS-003 – Fluorescence Scanning

Whole Slide Imaging using Multiple Fluorescence Channels

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Abstract

Whole Slide Imaging is a still young technology to digitally scan and archive tissue slides. With applications in digital pathology as well as in 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 all functional flexibility of a research microscope. In this study, we show that the MMI CellScan slide scanner, with its sensitive CMOS camera and its high position accuracy microscope stage, is able to capture and compile multi-color fluorescence images.

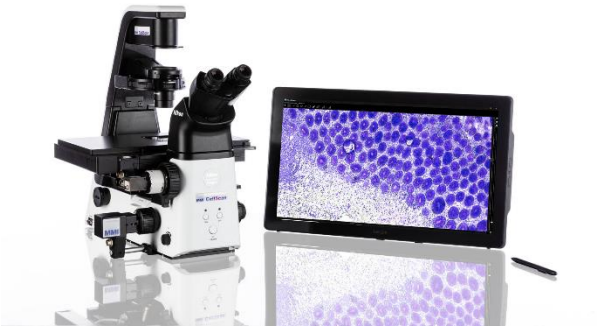

Figure 1: MMI CellScan system on the Nikon Ts2R inverted microscope. The system is compatible with many microscope brands and models and can be combined with all MMI cell isolation systems for various research applications.

Introduction

Whole slide imaging (WSI) or virtual microscopy is a technology to scan and digitally archive slides in high resolution. Slide scanners take separate images of each field of view across the entire microscopy slide. The individual pictures are then stitched together to generate a single image file in high resolution.

The technology of whole slide imaging developed rapidly over the last decade, since contemporaneously, storage of large datasets, barcoding and file tracing as well as data exchange were improving tremendously. Telepathology workflows emerged and thus pathologists are now able to consult experts on specific tissue types or diseases, or to discuss non-obvious cases with their colleagues from all over the world by using digital slides.

Furthermore, digital slides are not susceptible to quality loss such as color changes, photobleaching or degradation issues caused by long-term storage. By digitally archiving tissue slides researchers are able to analyze the tissue with same confidence after years and decades.

The MMI CellScan is a whole slide imaging system which is built on standard research microscopes. Therefore, this slide scanner

also can utilize all functionalities of the research microscope making this system highly versatile, flexible, and upgradeable. Scanning is not limited to one resolution, but changing objectives or switching between brightfield and fluorescence imaging modes can be performed with just one mouse click (if installed on a fully motorized microscope such as the Nikon Ti2E or the Olympus IX83). In addition to standard microscopy slides, the MMI CellScan is compatible with various sample formats such as well plates and dishes as well as MMI Membrane Slides for laser microdissection.

Here, we present the new MMI CellScan whole slide imaging system using fluorescent tissue sections. Digital images of single fluorescent channels are acquired and then automatically superposed to generate one multi-color image. The images are saved in the open BigTIFF format which is compatible with our free MMI CellViewer as well as many other slide viewer 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 4 x. The software automatically identified the region where tissue was present. A focus map has been automatically assigned by the MMI CellScan software. The pre-defined tissue area was then scanned at a 20 x magnification (CFI S Plan Fluor ELWD 20xC objective) using the pre-selected DAPI and FITC channels to be able 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 be able 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 is able to scan a 15 mm x 15 mm section using the 20 x objective in less than 1 min 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 NA means the system is not limited. The image was then automatically superposed and saved in a pyramid BigTIFF file format.

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

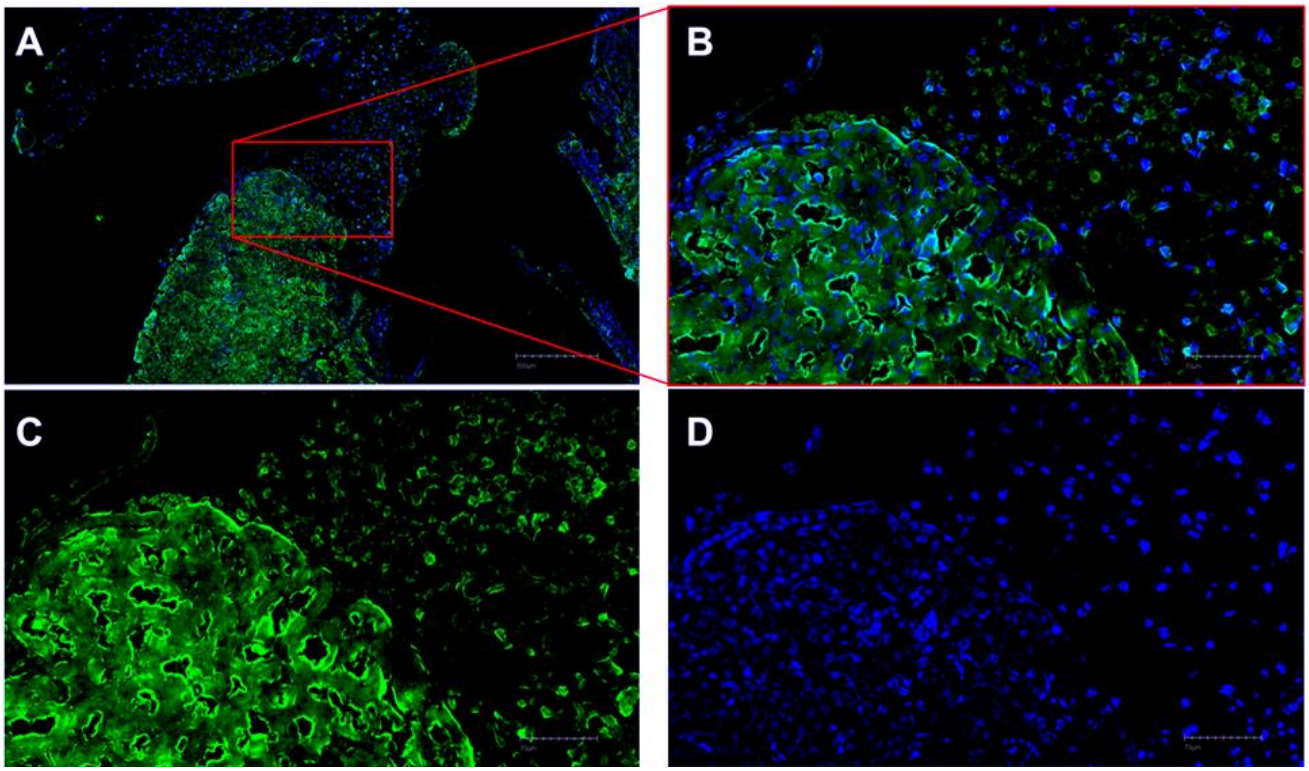

Figure 2: Image analysis using the free MMI CellViewer. The image opens in the lowest magnification to fit the window size. Seamless and rapid zooming down to the full resolution is enabled by a binning algorithm. A) Overview image of the tissue section at low magnification. Both channels DAPI (blue) and CF 594 (green) are shown. B) Zooming into the 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.

As the MMI CellScan is able to accurately save 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 reduces exposure of the sensitive sample to UV light and thus minimizes photobleaching effects.

Discussion

In this study, we demonstrate that the MMI CellScan is a high performance slide scanner which is able to scan 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 saved in an open file format.

A key benefit of acquiring a whole-sample image is to reduce sampling bias which can occur when only acquiring selective areas. Therefore, it will increase the robustness of the data and the validity of experiment.

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

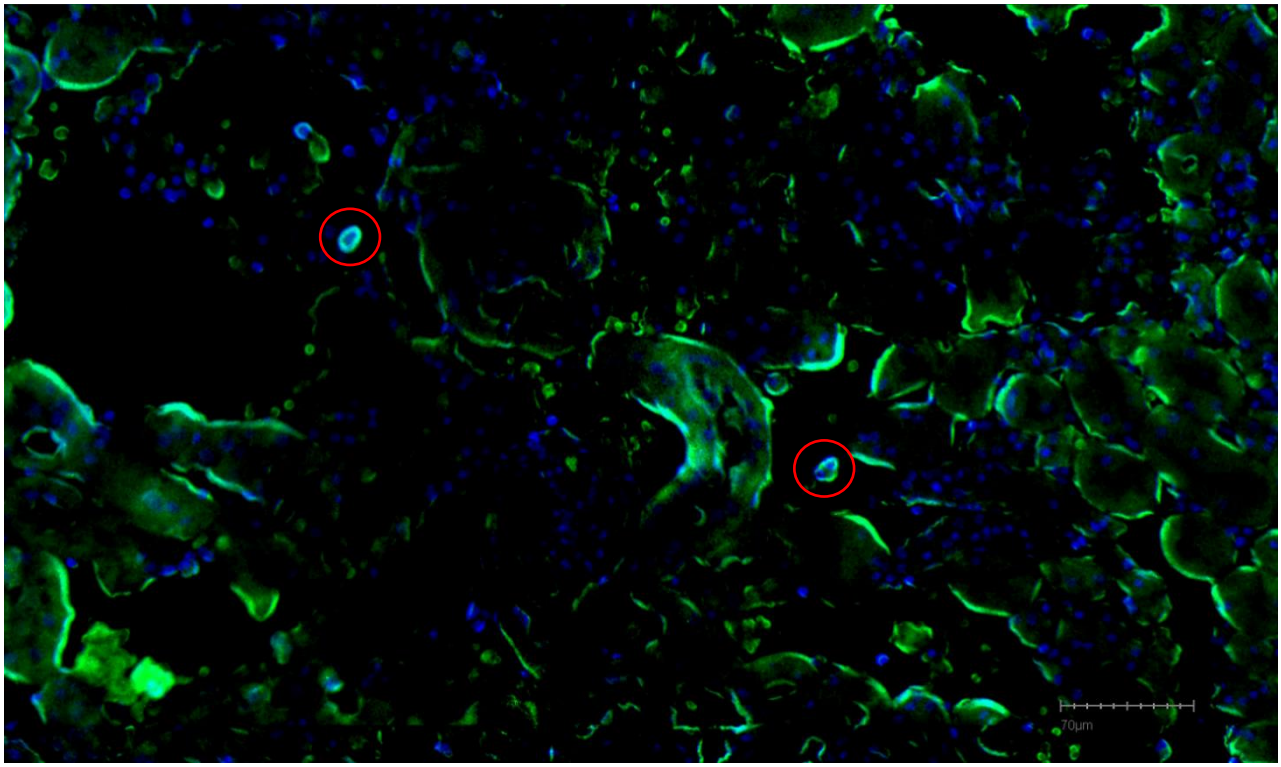

Figure 3: Overlay image shows accurate positioning with highlighted (red circles) structures.

In addition to standard consumables, the MMI CellScan, can scan MMI Membrane Slides dedicated for Laser Microdissection. Interestingly, the MMI CellScan can be integrated on the MMI CellCut system to uniquely and seamlessly combine whole slide imaging and laser microdissection. This workflow reduces light exposure of the sensitive sample to the scanning time and thus minimizes photobleaching effects. In addition, the scanned image files can be shared with colleagues and collaboration partners including all the labels and annotations.

The MMI CellScan is thus a highly versatile and flexible scanning system providing useful features and options for optimal scanning of any sample type in brightfield and fluorescence.