Application Note MMI-CS-004 –Scanning and Microdissection

Next Generation Laser Microdissection: Whole Slide Imaging meets Laser Microdissection

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Abstract

The MMI CellCut Laser Microdissection system and the MMI CellScan Whole Slide Imaging system are both integrated on research microscopes and fully compatible with common objectives and imaging modes.

In this study, we show that the MMI CellScan can be combined on one microscope platform together with the MMI CellCut. Importantly, whole slide scanning can be uniquely integrated into the laser microdissection workflow. The Next Generation Laser Microdissection comprises image scanning and analysis, as well as cell selection and laser cutting in one intuitive software interface within the MMI CellTools platform.

Introduction

Laser microdissection is widely used to selectively cut and isolate single cells or tissue areas from tissue sections with applications in various research areas such as oncology, pathology, immunology, forensics, and crop science [1-5]. Laser microdissection therefore allows downstream analyses, such as RNA sequencing, from an individual cell or very specific set of cells rather than from a heterogeneous mixture.

Whole slide imaging (WSI) - or virtual microscopy – is an emerging technology to digitally scan and archive slides in high resolution. Scanners acquire images of each field of view across the entire microscopy slide. The individual images are stitched together to generate one single digital image in high resolution.

Figure 1: MMI CellScan and MMI CellCut system on the Olympus IX83 inverted microscope. The systems are compatible with many microscope brands and models for various research applications.

Since the original tissue section will be segmented during laser microdissection, it is desirable to digitally archive tissue slides for full documentation and to be able to analyze the tissue together with the molecular data

after years and decades. Thus, we combined the MMI CellScan and MMI CellCut on one microscope.

Here, we demonstrate that the new MMI CellScan whole slide imaging system can be combined with laser microdissection for full documentation of native and dissected tissue sections. In addition, we show that slide scanning and laser microdissection can be integrated in a novel workflow enabling more remote work, fuller utilization of equipment, and less hands-on time at the instrument.

Material and Methods

An FFPE tissue section was mounted onto an MMI Membrane Slide (Product number 50102) and covered with a standard microscope slide. The slide was placed into the 3-slide holder on the stage of an inverted microscope outfitted with the MMI CellCut and MMI CellScan module.

Using the Preview Scan function, the slide was rapidly pre-scanned at a magnification of 4 x. The software autodetection algorythm identified the regions where tissue was present and then scanned those areas at 20 x magnification. A focus map which assures perfect focus despite topological changes in tissue. This can be automatically assigned or applied manually. The image was automatically saved as BigTIFF and was analyzed using the free MMI CellViewer software (Figure 2).

The scanned image was analyzed and target cells were identified. The target cells were marked and the shapes were exported into an XML-file. This can be done remotely and the small XML file emailed between collaborators. The XML-file was transferred to the laser microdissection microscope and opened in MMI CellTools for laser microdissection.

Laser microdissection was conducted using the MMI CellCut. Settings for optimal cutting (laser focus, power and speed) were adjusted on the same tissue section in a non-target area.

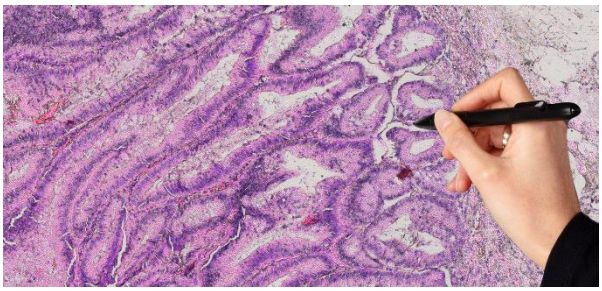

Figure 2: The MMI CellViewer allows to mark and annotate cells and tissue areas from any desktop PC. The selected shapes can be saved and transferred to the live image at the microscope for laser microdissection.

The area of interest was transferred to the live view via XML-file import. After laser cutting, the excised target cell was taken up by an adhesive 0.5 ml MMI Isolation Cap (Product number 50204). The success of isolation was verified by focusing onto the surface of the isolation cap.

Results

In this study, we evaluate the unique combination of laser microdissection and whole slide imaging. In addition to full documentation and digital archiving of tissue sections, this novel workflow offers further options for remote work.

The tissue sections mounted on MMI Membrane Slides were scanned using the MMI CellScan module. The MMI CellTools software offers different features and parameter settings to efficiently scan one or several slides automatically providing optimal image quality both on standard glass slides and on MMI Membrane Slides. With the sensitive CMOS MMI camera and the fast microscope 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. The image was then automatically saved in a standard BigTIFF file format.

The scanned images were viewed and analyzed at a remote PC equipped with 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. Easy navigation tools enable fast screening through the sample file.

Target cells can be directly identified, selected, marked and annotated in the scanned image. The selected areas can then be transferred to the live image at the microscope via XML-files. Because of the precise microscope stage, positions within the scanned image are accurately determined and are not compromised by stitching algorithms. Therefore, after transfer of the shapes, the target cells can readily be cut using the MMI CellCut laser microdissection module.

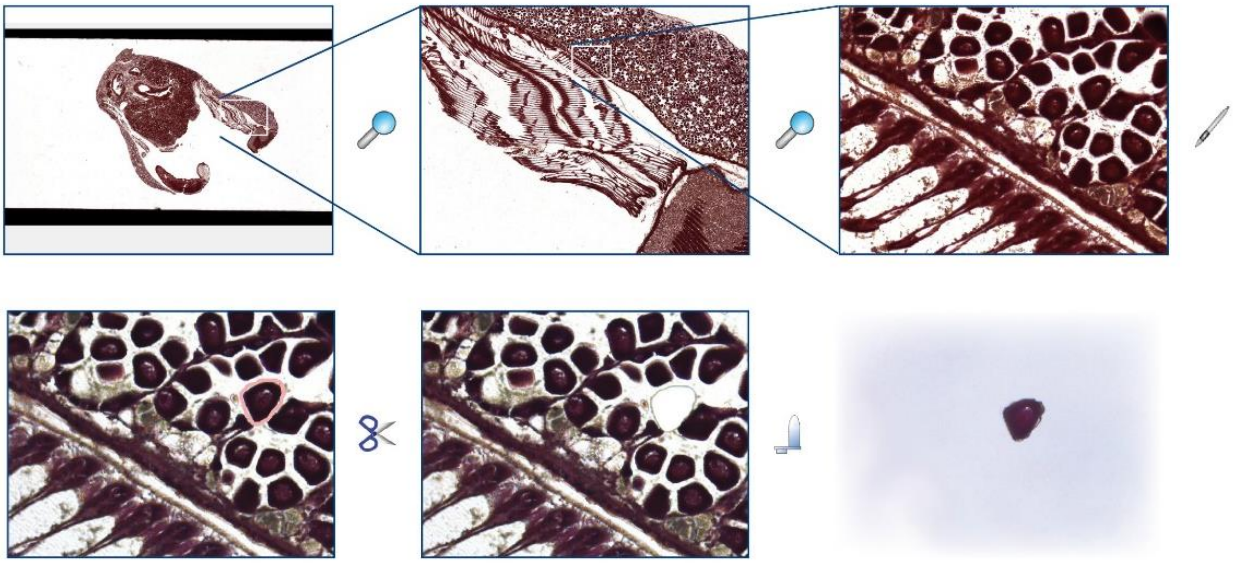

Figure 3: Image analysis using the free MMI CellViewer followed by laser microdissection using CellCut. The image opens in low resolution to fit the window size. Seamless and rapid zooming down to the full resolution is enabled by a binning algorithm. Areas of interest and single cells can be marked and annotated for subsequent cutting with laser microdissection. The MMI Isolation Caps with its adhesive surface takes up the excised target cell in a sterile, contamination-free way.

Discussion

In this study, we demonstrate that the MMI CellScan and the MMI CellCut can be installed on a single microscope to save money and lab space as well as to increase overall utility for the investment. The combination of laser microdissection and whole slide imaging, which we name “Next Generation Laser Microdissection”, offers several new possibilities and novel workflow options which have not been possible before.

After performing laser microdissection on a tissue section, the slide is typically discarded as the target cells have been isolated and separately analyzed using molecular biology methods. The original slide information therefore is lost. Now, using the MMI CellScan, the tissue section can be archived digitally in full resolution before and after cutting. Therefore, this combination of methods enable comparative analyses with molecular data even after years and decades. Furthermore, the spatial information as well as

the cellular micro-environment add further dimensions to the obtained research data.

Since the scanned images can be viewed and analyzed at any computer equipped with the free MMI CellViewer software, pathologists and researchers can access the scanned images anytime and from any place. Thus, it is no longer required to sit at the microscope for hours, or book precious microscope time, but image analysis can be performed remotely from the office or even from home. Moreover, highly experienced researchers and clinicians can save time as slide scanning and subsequent laser microdissection can now be performed by lab staff and students with only minimal training.

Since the MMI CellScan and MMI CellCut are based on standard research microscopes, they also can utilize all functions of the research microscope making this system highly versatile, flexible, and upgradeable. Scanning and cutting is not limited to one resolution - 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).

The MMI CellScan fulfills all the needs of a standard slide scanner. It can be employed to scan full resolution slides in a medium-throughput setting and in combination with the MMI CellCut laser microdissections offers new workflow options. Thus, Next Generation Laser Microdissection includes fully documented and high content research, and additionally enables remote work anytime and anyplace.

To access the video on Next Generation Laser Microdissection, please scan the QR code:

References

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