WHOLE-SLIDE IMAGING

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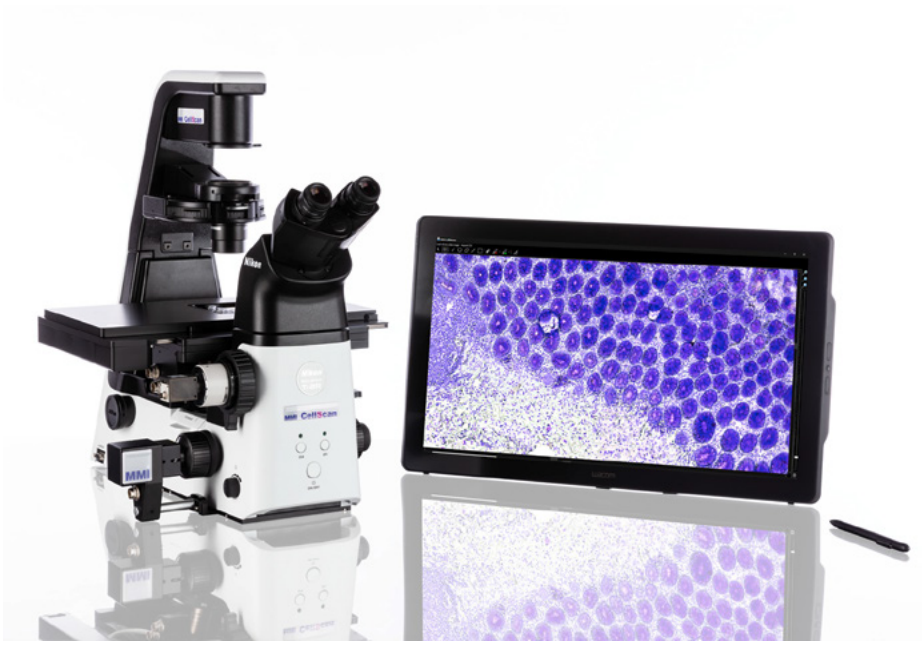
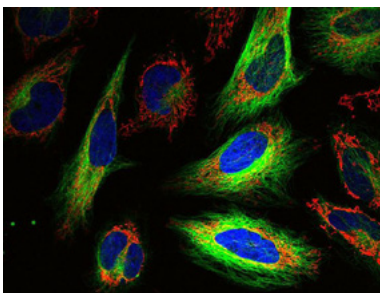
MMI

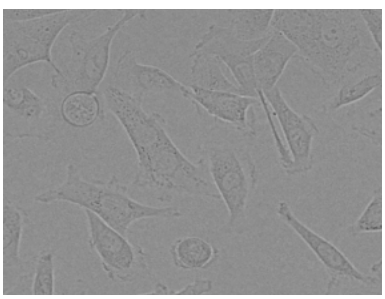
MMI CellScan

Whole Slide Imaging and Beyond:

A new open platform for scanning & bio-imaging







- perform 5D Whole Slide Imaging: X, Y, Z-stacks, multi-color fluorescence and time-lapse
- scan any biological sample
- stay flexible with the open BigTIFF file format
- install unlimited FREE copies of the MMI CellViewer Software wherever you need it

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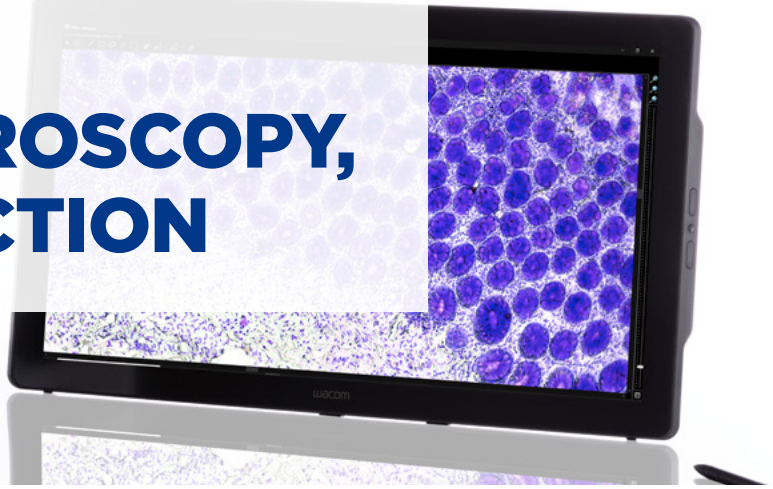
VIRTUAL MICROSCOPY, AN INTRODUCTION



Whole-slide imaging (WSI)—also known as virtual microscopy—is becoming increasingly popular in digital pathology as well as in clinical and basic research. Scanners take images of each field of view across an entire conventional microscopy slide. The pictures are then stitched together to generate a single, high-resolution digital image that researchers use for various purposes.

Solutions for Operational Sustainability

In the 1990s, methods and software systems arose from computer science spatial dataset research that allowed for whole-slide imaging.[1] The research began when scientists needed to analyze, visualize, and query sensor data acquired from satellites and other basic and applied earth science applications. Inspired by this work, a research team at the University of Maryland led by Joel Saltz developed the earliest software—the Active Data Repository (ADR) system—for retrieving and processing large amounts of spatio-temporal data in 1996. The group then developed software necessary to support the earliest "virtual microscope" for whole-slide imaging between 1996 and 1998.[2] Meanwhile, James Bacus created the first commercial slide scanner called the BLISS (Bacus Laboratories Inc. Slide Scanner) system in 1994.[3]

The first digital microscope systems took more than 24 hours to scan a single slide. However, ongoing technical advances in optical imaging technologies now allow scientists to rapidly generate large quantities of microscopy information. Equally dramatic improvements in storage and computational technology allow researchers to process large WSI datasets. As a result, WSI devices have become important tools for supporting routine diagnostics work and scientific discovery.[1]

Why Whole-slide Imaging?

WSI technology offers many advantages over conventional microscopy, such as portability, ease of sharing, retrieval of images, workload balancing, and image analysis. Scientists use WSI in many clinical and nonclinical settings. Clinical scientists are particularly excited about its use in digital pathology, where published data shows excellent correlation between diagnoses made with WSI and conventional light microscopy.[4] The US Food and Drug Administration (FDA) permitted marketing of WSI systems for pathological diagnoses in April 2017. Unsurprisingly, several pathology labs have since successfully gone fully digital.[1]

Drug discovery is another rapidly expanding area for WSI technology. Many biotech and pharmaceutical companies have adopted the technology, enhancing researchers' ability to understand and work with model organisms. WSI helps reduce costs and improve standardization and data management in research and development. Additionally, WSI facilitates machine learning in the drug discovery process.[5]

In precision medicine, WSI helps scientists develop personalized drugs by allowing data sharing with a wide community of scientists, enabling clinicians and researchers to understand disease behavior at the individual patient level. WSI research also benefits from artificial intelligence, which enables scientists to mine subvisual morphometric phenotypes to improve patient management.[6] Researchers in many fields find advantages to using whole slide digital images for microscopic specimen examination. The data are easily duplicated; they do not break or deteriorate; they can be stored and catalogued with ease; and they are easily sharable with collaborators worldwide.

Reference

1) L. Pantanowitz et al., "Twenty years of digital pathology: an overview of the road travelled, what is on the horizon, and the emergence of vendor-neutral archives," *J Pathol Inform*, 9:40, 2018.
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4) A. Saco et al., "Validation of whole-slide imaging for histolopathogical diagnosis: Current state'" *Pathobiology*, 83:89-98, 2016.
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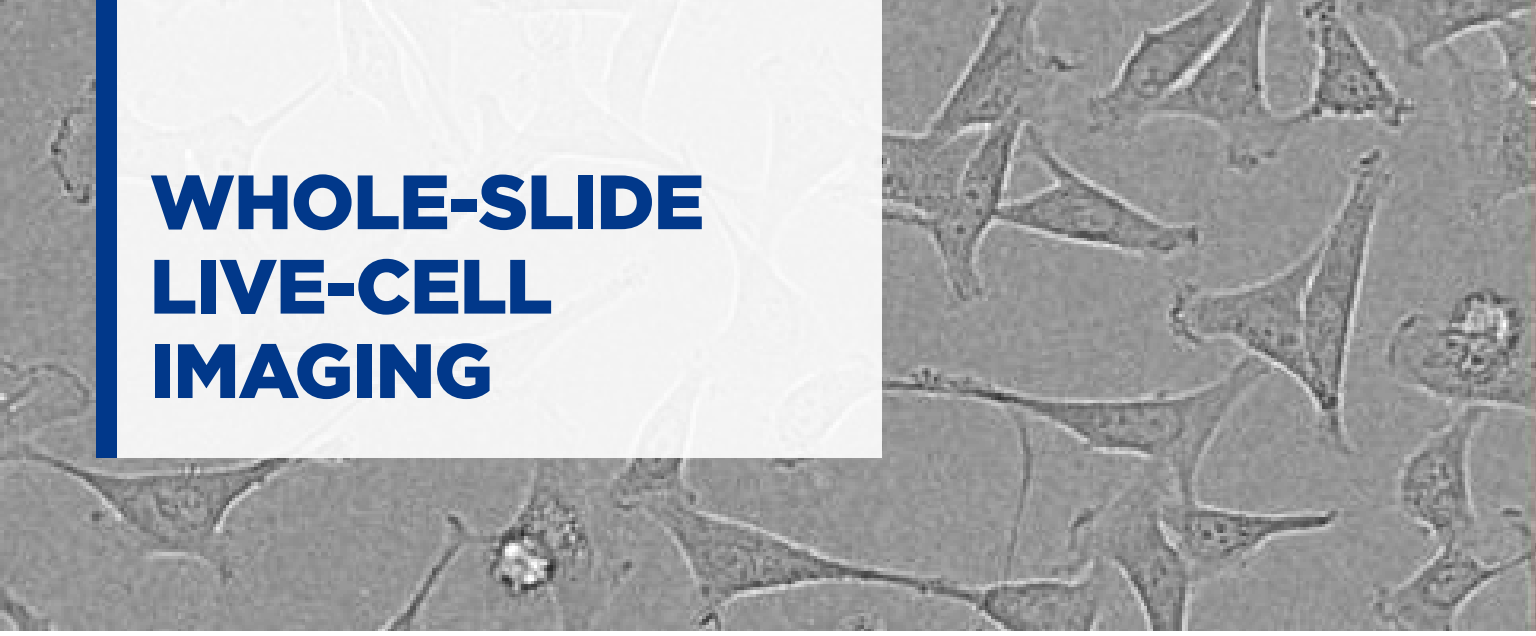

WHOLE-SLIDE LIVE-CELL IMAGING

Live-cell imaging involves time-lapse microscopy on living cells. Scientists use it to better understand biological function and cellular dynamics. The field has expanded greatly in recent years after the advent of new technology that maintains cells in physiological conditions throughout the imaging process. Scientists can use Molecular Machines & Industries' (MMIs) CellScan whole-slide imager in combination with a stage top incubator to perform live-cell imaging.

Live-cell Imaging Applications

Cell biologists increasingly rely on live-cell imaging to observe cell-cell interactions, the behavior of single cells, and cell organelle or cellular molecule dynamics. Phase contrast microscopy, fluorescence and confocal microscopy, multiphoton microscopy, light sheet microscopy, and super-resolution microscopy enable them to measure cell proliferation over time,[1] investigate cell migration in chemotaxis assays,[2] and gain insight into cytoskeletal dynamics.[3]

WSI Live-cell Imaging Workflow

A typical integrated live-cell imaging workflow with WSI consists of the following:

- Cultivate cells and transfer them to the reservoirs of a µ-slide 8-well chambered coverslip (Cat. No. 80826 at ibidi; Product No. 50114 at MMI). Grow the cells for 24 hours.
- Transfer the cells to the MMI CellScan equipped with a properly equilibrated ibidi Stage Top Incubation System (i.e., 37 °C, 10 % CO_2, 70% humidity for HeLa cells).
- Scan at an appropriate magnification (i.e., 20x for HeLa cells) with the time-lapse function enabled on the MMI CellScan.
- CellScan will automatically capture images depending on the settings selected (i.e., one image every 30 minutes over a 24-hour period).
- During scanning, the software will automatically stitch together single field of view pictures to form one full-resolution image per timepoint.
- Analyze images using the free MMI CellViewer or other software.

Further Cultivate Cells after Live-cell Imaging

MMI CellScan saves images with precise information, so scientists can monitor individual cells, and cells remain viable after live-cell imaging with MMI CellScan. Researchers can further culture the cells, or subject them to single-cell isolation procedures facilitated by the MMI CellEctor, which is able to select single adherent cells from a µ-slide 8 well. Scientists can then subject individual cells to further analysis, such as transcriptomics, proteomics, or sequencing.

Reference

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CAPTURING AND COMPILING MULTICOLOR FLUORESCENCE IMAGES

The CellScan system is based on standard research microscope technology, so scanning is not limited to one resolution. Researchers can change objectives or switch between brightfield and fluorescence imaging modes with just one mouse click. Scientists can collect digital images of single fluorescent channels and automatically superpose them to generate one multicolor image file.

Fluorescence Imaging Applications

Fluorescence microscopy is a powerful tool in the life sciences. Scientists regularly use fluorophores to label samples. Fluorophores are available to emit light in virtually any color, allowing scientists to obtain a clear image of almost any structure or area of interest in a cell.

WSI works well with fluorescence imaging applications. Digital slides are not susceptible to quality loss such as color changes or degradation caused by long-term storage. By digitally archiving tissue slides, researchers can analyze the tissue with the same confidence after years and decades.

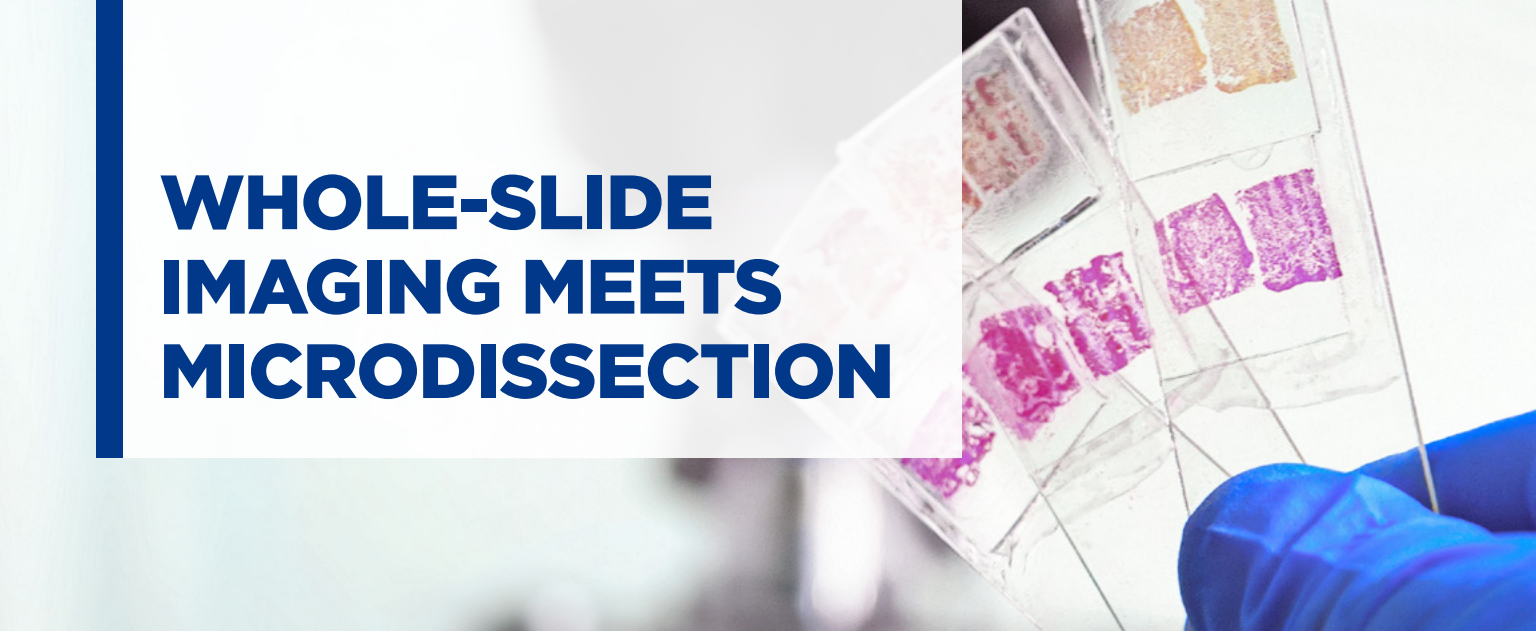
Typical Fluorescence Microscopy WSI Workflow

Formalin-fixed paraffin-embedded (FFPE) tissues are common sources of archived material, especially for cancer research. A typical fluorescence microscopy workflow with WSI using a triple-fluorescently stained FFPE tissue section consists of the following steps:

- Mount the FFPE tissue section onto an MMI Membrane Slide covered with a standard microscope slide.
- Place the slide into the slide holder on the stage of an inverted microscope outfitted with the MMI CellScan module.
- Scan at a preliminary magnification (i.e., 4x) using the Preview Scan function.
- The CellScan software automatically identifies regions containing tissue and scans those areas at an appropriate magnification (i.e., 20x); this autofocus function automatically sets reference points in the z-dimension to optically flatten uneven samples.
- Save the image automatically as a BigTIFF file and analyze it using the free MMI CellViewer or other software.
- The software will automatically repeat these steps for pre-selected florescent channels. The software platform offers additional features and parameter settings for efficiently scanning slides in one or more fluorescence imaging modes.
- Use MMI CellViewer software to automatically digitally superpose images into one multicolor image file.

BigTIFF is an open file format that is compatible with CellViewer and many other slide viewer and image analysis software packages. Using the MMI CellExplorer software tool, scientists can also interrogate images and automatically detect target cells based on their fluorescence signals. Target cells can then be isolated using the MMI CellCut or MMI CellEctor system, which can be integrated on a single microscope platform.

WHOLE-SLIDE IMAGING MEETS MICRODISSECTION



Researchers from a multitude of fields use microdissection to selectively isolate single cells or tissues. The technique enables downstream analyses such as RNA sequencing. Scientists often discard the larger cut tissue section, losing valuable information from the original slide. However, MMI's CellScan, in combination with the CellCut Laser Microdissection system, allows researchers to image a tissue section and then precisely select cells and dissect the tissue while concurrently preserving information about the original uncut tissue and position information about the cut.

Tissue Micromanipulation in Research

Laser microdissection enables researchers to selectively cut and isolate single cells or tissue areas from tissue sections. Researchers in oncology, pathology, immunology, forensics, and crop science benefit from the technique since it allows for downstream analyses, such as RNA sequencing, from an individual cell or precise selection of cells.

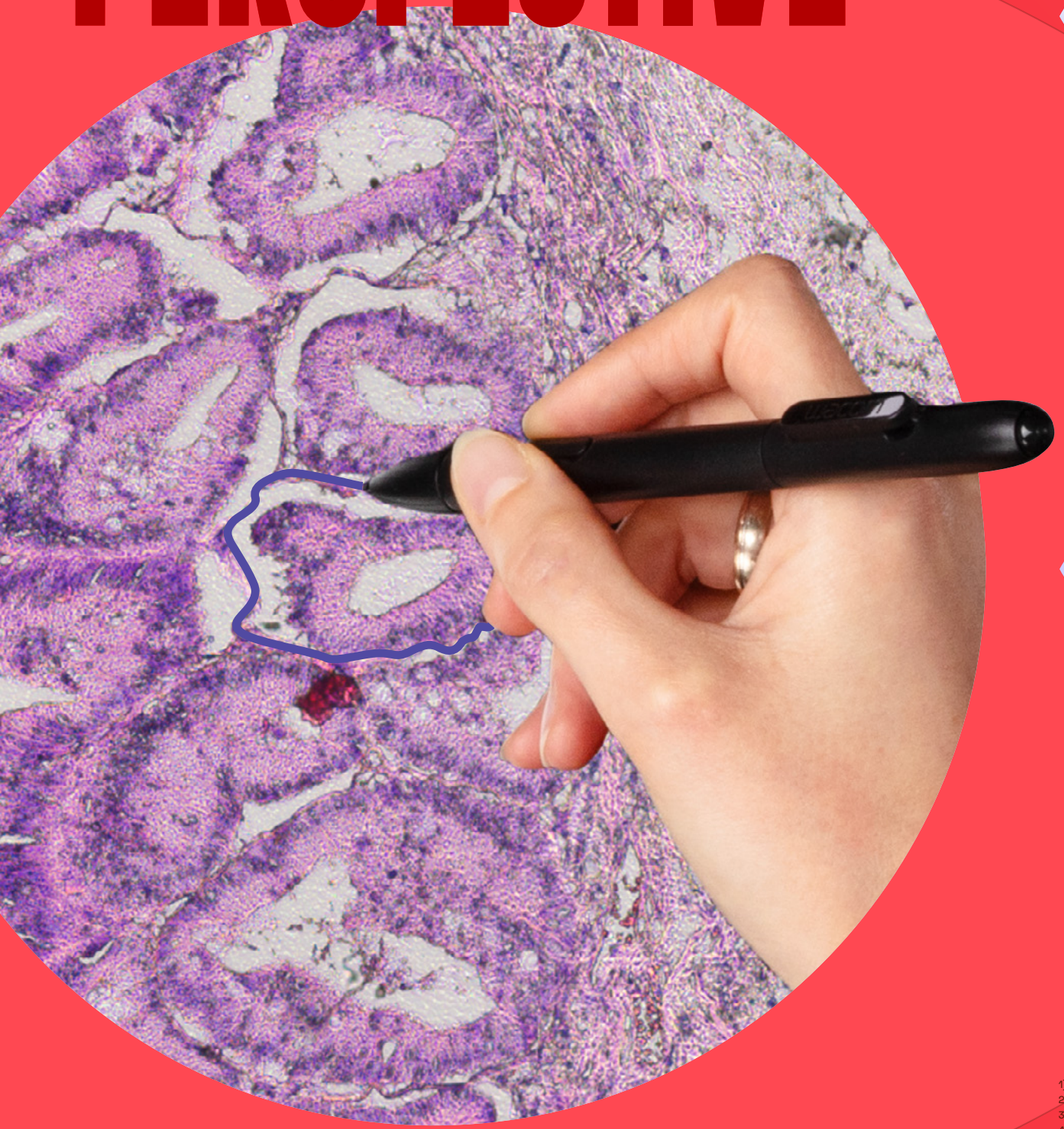
Typical Microdissection with WSI Workflow

Scientists commonly use microdissection for FFPE histology specimens in cancer research, where the recovered cells are studied with a variety of DNA, mRNA, and protein analysis methods to expansively examine the molecular anatomy of cells in tissue sections. A typical microdissection workflow with WSI using FFPE tissue section consists of the following steps:

- Mount the FFPE tissue section onto an MMI Membrane Slide and cover it with a standard microscope slide.
- Place the slide into a slide holder on the stage of an inverted microscope outfitted with the MMI CellCut and MMI CellScan module.
- Scan at a preliminary magnification (i.e., 4x) using the Preview Scan function.
- The CellScan software automatically identifies regions containing tissue and then scans those areas at an appropriate magnification (i.e., 20x); this autofocus function automatically sets reference points in the z-dimension to optically flatten uneven samples.
- Automatically or manually assign a focus map.
- Save the image automatically as a BigTIFF file and analyze it using the free MMI CellViewer or other software.
- The MMI CellTools software offers different features and parameter settings to efficiently scan one or several slides automatically, providing optimal image quality on standard glass slides and on MMI Membrane Slides. With the sensitive CMOS MMI camera and the fast microscope stage, the MMI CellScan can scan a 15 mm x 15 mm section using the 20x objective in less than 1 min.
- Analyze the scanned image and identify target cells.
- Mark the target cells and export the shapes into an XML-file. This step can be performed remotely and the small XML-file may be emailed between collaborators.
- Transfer the XML-file to the laser microdissection microscope and open in MMI CellTools for laser microdissection.
- Conduct laser microdissection on the MMI CellCut. Adjust the settings for optimal cutting (i.e., laser focus, power, speed) using a non-target area on the same tissue section.
- Excise the target cell and place it into an adhesive MMI Isolation Cap.
- Verify successful isolation by focusing on the surface of the isolation cap.

Combining laser microdissection with WSI significantly reduces hands-on time at the instrument. With the added security of fully supported remote workflows, researchers can be confident that they are getting the most reliable information from their samples.

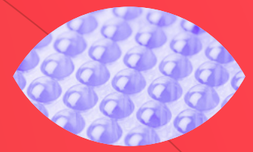
A Bird's Eye PERSPECTIVE



Scientists use whole-slide imaging (WSI) in various research areas such as pathology, oncology, and neuroscience. WSI's ability to create shareable and long-lasting digitized slide images transforms workflows and analyses.

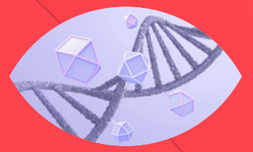
PATHOLOGIC DIAGNOSES

Traditionally, pathology relied almost solely on specimens mounted on glass slides. As such, initial diagnoses were often delayed while waiting for the glass slide or specimen to be physically delivered to the appropriate pathologist. WSI allows scientists to scan the entire glass slide and use machine learning (ML), deep learning (DL), and artificial intelligence (AI) tools for diagnostics. WSI is approved by the Food and Drug Administration for primary diagnoses in the United States of America.[1]



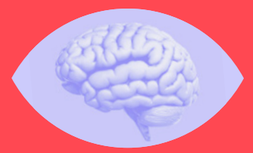
CORRELATING PHENOTYPES WITH MOLECULAR DATA

Pathologists typically review tumor morphology in tissue sections to classify and grade cancer, however, human review may result in low reproducibility and inter-observer disagreement. WSI can partially overcome these shortcomings by quantitatively and reproducibly measuring tissue structures on a large-scale. Scientists can use large-scale morphologic analysis of pathology images to correlate phenotypic groups with molecular data and clinical outcomes.[2]



LINKING EPIGENETIC AND MORPHOMETRIC FEATURES

Researchers use WSI to investigate the interaction between cancer and DNA methylation profiles to provide a better understanding of tumor pathobiology. Classical machine learning algorithms associate the DNA methylation profiles of cancer samples with morphometric features extracted from whole slide images.[3]



MAPPING MULTICOLOR FLUORESCENT MARKERS IN BRAIN TISSUE

WSI is ideally suited for screening whole brain tissues for rare physiologically relevant events, such as microhemorrhages, which may be missed using conventional microscopy. Scientists mount large brain sections on large format slides. Slide scanners that can handle larger formats and image multiple fluorescent channels have greatly expanded the utility of digital pathology image analysis within the field of neuroscience.[4]

STREAMLINING MICRODISSECTIONS

Molecular diagnostic assays based on next-generation sequencing and proteomics require improved methodologies for procuring target cells from tissue sections. Laser microdissection can successfully isolate distinct cells from tissue specimens based on visual selection for many research and clinical applications. Large projects requiring molecular analysis of several cells or evaluation of numerous specimens can be daunting. WSI, in combination with microdissection, streamlines these processes, potentially speeding up clinical and life science microdissection workflows.[5]

DRUG DEVELOPMENT

Scientists performing tissue-based biomarker discovery research readily adopted digital pathology for the valuable and multiparametric outputs offered by quantitative image analysis. In combination with tissue microarrays, WSI allows researchers to compare potential biomarkers across all specimens simultaneously.[4]

1) A.V. Parwani, "Next generation diagnostic pathology: use of digital pathology and artificial intelligence tools to augment a pathological diagnosis," Diagn Pathol, 14:138, 2019.
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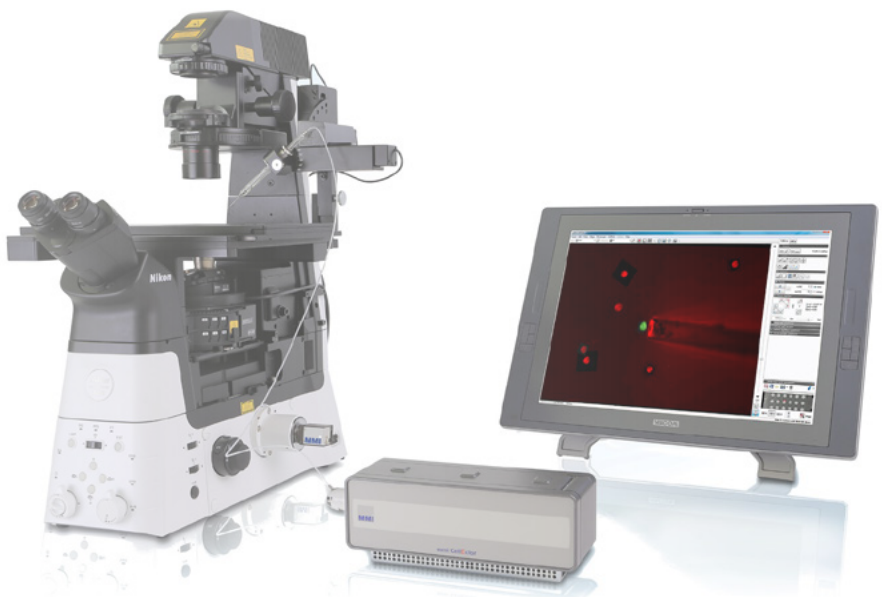
Microdissection - Micromanipulation - Imaging

MMI

Precision Technology for Imaging & Single Cell Isolation

- **Whole Slide Imaging for slide scanning and bioimaging with MMI CellScan**
- **Laser “Cap-sure” microdissection to isolate cells in tissue with MMI CellCut**
- **Capillary-based selective isolation of single cells with MMI CellEctor**
- **Optical tweezers to quantify biological forces with MMI CellManipulator**



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