Application Note

Cell Analysis

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Efficient Slide-Based Tissue Imaging for Research Workflows

Fluorescence, color brightfield, and targeted ROI acquisition with the Agilent BioTek Cytation 9 cell imaging multimode reader

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Abstract

This application note demonstrates slide-mounted tissue imaging with the Agilent BioTek Cytation 9 cell imaging multimode reader. H&E-stained and fluorescent tissue sections, as well as a tissue microarray were imaged using fluorescence and color brightfield acquisition. The examples show how automated calibration, laser autofocus, high-numerical-aperture objectives, and region-of-interest (ROI)-based acquisition can support efficient, research-scale slide imaging while preserving tissue-level context and cellular-level detail.

Introduction

Tissue-based imaging helps researchers connect cellular mechanisms, biomarker expression, and tissue architecture with assay outcomes. This context is especially useful when studies move across microplate-based assays, live- and fixed-cell imaging, and slide-mounted tissue samples. In these workflows, slide imaging adds value when it fits alongside existing experimental systems and provides clear tissue context without adding unnecessary workflow complexity.

The Cytation 9 cell imaging multimode reader brings slide imaging into an automated microscopy environment that can also support cell- and assay-based applications. It combines fluorescence microscopy, color brightfield imaging, high-resolution objectives, and microplate-based imaging capabilities in one system. This application note evaluates several slide-mounted sample types to demonstrate image quality, acquisition efficiency, and the practical use of targeted ROI imaging for tissue sections and arrayed tissue samples.

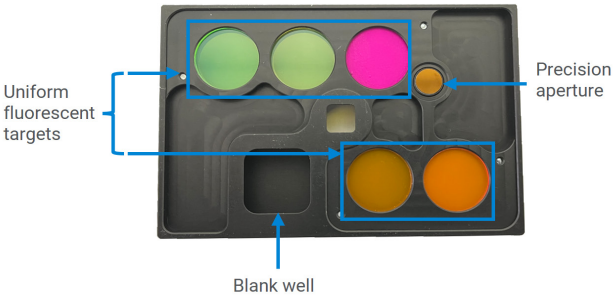
Experimental

Slide-mounted fluorescent and color brightfield samples were imaged using the Cytation 9 automated imaging system. The system was configured with high-numerical-aperture apochromat objectives, laser autofocus, fluorescence filter sets, and color brightfield illumination. Agilent BioTek Gen5 software for imaging & microscopy was used for image acquisition, automated calibration, overview imaging, ROI detection, and stitched image generation.

Before sample acquisition, the automated calibration routine was used to perform optical calibration, flat-field correction, and white balancing. The calibration plate contained a precision aperture of 100 µm and five uniform fluorescence intensity targets. Calibration images were acquired for each objective and color combination, and image corrections were applied automatically during subsequent acquisitions (Figure 1).

Samples included fluorescent mouse colon and kidney tissue sections, H&E-stained mouse embryo and human kidney sections, and a 72-core H&E-stained human normal organ tissue microarray. Tissue location was identified using low-magnification overview imaging. High-magnification images were then acquired using 20x or 40x apochromat objectives, with laser autofocus used to determine the focal plane from the coverslip reflection. For ROI-based workflows, the Agilent BioTek Gen5 automatic region-of-interest (AutoROI) module was used to identify tissue-containing regions and exclude empty slide areas from high-magnification acquisition.

A



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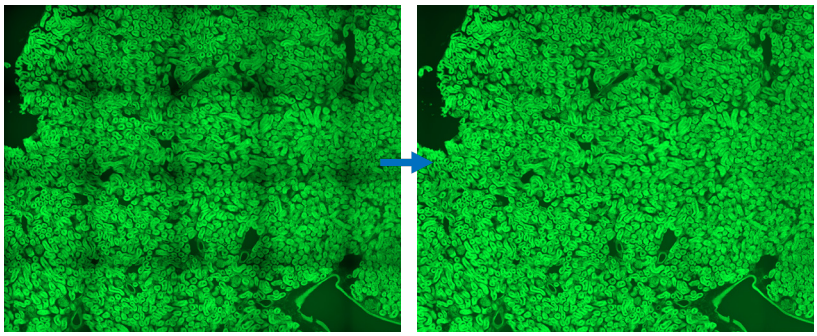

C

	20x Apochromat	40x Apochromat
Numerical aperture	0.8	0.95
Resolution (nm)	279	235
Working distance (mm)	0.6	0.2
Field of view (mm)	0.7 x 0.7	0.35 x 0.35

D

Figure 1. Agilent BioTek Cytation 9 cell imaging multimode reader features for automated slide imaging. (A) Precision calibration tool for automatic stage alignment and illumination correction. (B) Correction is automatically applied during montage acquisition to eliminate tiling artifacts in fluorescence and color brightfield imaging modes. (C) High-resolution Olympus UPLXAPO 20x 0.8 NA and UPLXAPO 40x 0.95 NA objectives with widefield of view camera. (D) Cytation 9 slide capacity is scalable to 120 microscope slides with the compatible Agilent BioTek BioStack microplate stacker.

Results and discussion

Multiplex fluorescence tissue imaging

Multiplex fluorescence imaging enables researchers to examine how biomarkers, cell types, and structural features are organized within intact tissue. A 100 µm thick mouse colon section (Mouse Colon, 4-color, OptiSlides; Luxidea; Calgary, AB, Canada) was imaged in four fluorescence channels to visualize nuclei, cytoskeleton, neurons, and blood vessels (Figure 2A).

A 4x overview image was used to identify the tissue region, followed by high-magnification imaging with 20x and 40x apochromat objectives in the DAPI, GFP, TRITC, and Cy5 channels. The 20x acquisition covered a 2.2 mm^2 sample area with 48 images in 46 seconds. The 40x acquisition generated 252 images in 4 minutes 30 seconds (Table 1).

A 16 µm thick mouse kidney section (FluoCells Prepared Slide #3; Thermo Fisher; Waltham, MA, USA) was imaged in three fluorescence channels to visualize nuclei, plasma membrane, and cytoskeleton (Figure 2B). A 4x DAPI overview image was used to identify the tissue region before high-magnification imaging with a 20x apochromat objective in the DAPI, GFP, and TRITC channels. The final image set contained 819 images and covered a 63 mm^2 sample area in just under 14 minutes (Table 1). Across these examples, the stitched images provide both broad tissue architecture and cellular-level detail, supporting evaluation of marker localization and tissue organization within a single workflow.

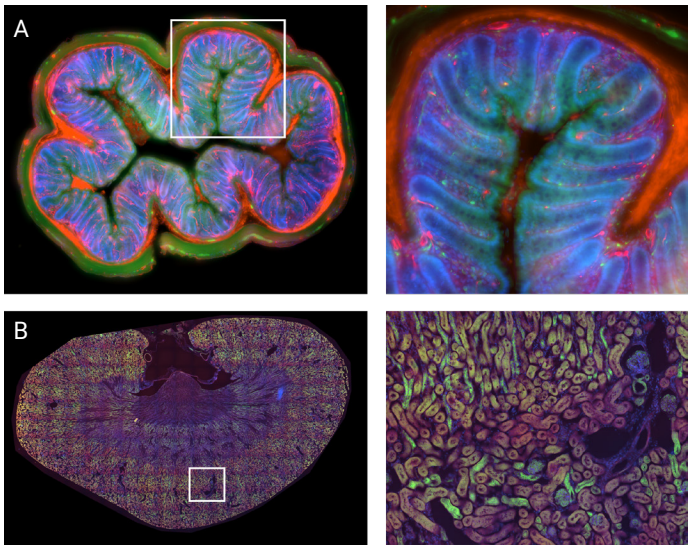

Figure 2. Multichannel immunofluorescence imaging of prepared tissue slides. (A) Mouse colon section at 40x with four fluorescent markers: nuclei (DAPI), neuron (GFP), cytoskeleton (TRITC), and blood vessels (Cy5). (B) Mouse kidney section at 20x with three fluorescent markers: nuclei (DAPI), wheat germ agglutinin (GFP), and actin (TRITC).

Table 1. Sample and slide imaging acquisition details.

	Mouse Colon	Mouse Kidney	Human Tissue Microarray	Mouse Embryo	Human Kidney
Staining	Nuclei DAPI Neurons Dylight 488 Vimentin Dylight 550 Blood vessels Dylight 649	Nuclei DAPI WGA Alexa Fluor 488 Phalloidin Alexa Fluor 568	H&E	H&E	H&E
Number of wavelengths	4	3	**3**	**3**	**3**
Sample area	2.2. mm^2	63 mm^2	**156 mm^2**	**60 mm^2**	**39 mm^2**
Magnification	20x	20x	**20x**	**20x**	**20x**
Total number of images	**48**	**819**	**5841**	**615**	**1230**
Acquisition time (minutes)	**0.8 min**	**13.7 min**	**37.9 min**	**7.5 min**	**5.1 min**
Normalized time (10 x 10 mm)	**36 min**	**22 min**	**24 min**	**13 min**	**13 min**

Color brightfield histology imaging

Color brightfield imaging remains important for assessing tissue morphology and relating structural context to targeted marker information. Cytation 9 uses automatic white balancing and sequentially controlled red, blue, and green illumination to acquire true-color histology images at high resolution. This capability was evaluated using large-format H&E-stained tissue sections representing both animal and human samples.

An H&E-stained mouse embryo section (donated by local research institution) was imaged with a 20x apochromat objective (Figure 3A). The tissue region was automatically detected from a 4x overview image using Gen5 AutoROI module, allowing empty regions to be skipped during high-magnification acquisition. The acquisition time was 7.5 minutes. An H&E-stained human kidney section with chronic nephritis (Konus; Medley, FL, USA) was imaged using the same workflow (Figure 3C). The kidney section covered 38 mm^2 and was imaged at 20x magnification in just over 5 minutes (Table1).

The final stitched images preserved whole-section morphology while retaining cellular detail in the inset regions.

These results illustrate how color brightfield slide imaging can be incorporated into a broader microscopy workflow without separating histology imaging from related cell- and assay-based studies.

Tissue microarray imaging with targeted acquisition

Tissue microarrays are useful when many related tissue samples must be compared under similar staining and imaging conditions. A 72-core H&E-stained microarray was imaged using Gen5 AutoROI module (FDA human normal organ tissue; US Biomax, Inc.; Derwood, MD, USA). A 4x overview image was generated, and each tissue core location and area was automatically detected. High-magnification images of each tissue core were acquired using a 20x apochromat objective (Figure 3B).

The aggregate tissue area was 156 mm^2, excluding the empty regions between cores. Image acquisition time was approximately 30 seconds per core, totaling about 38 minutes (Table1). This workflow shows how targeted acquisition can make arrayed tissue imaging more practical by identifying relevant tissue regions, supporting consistent acquisition, and excluding empty areas between cores.

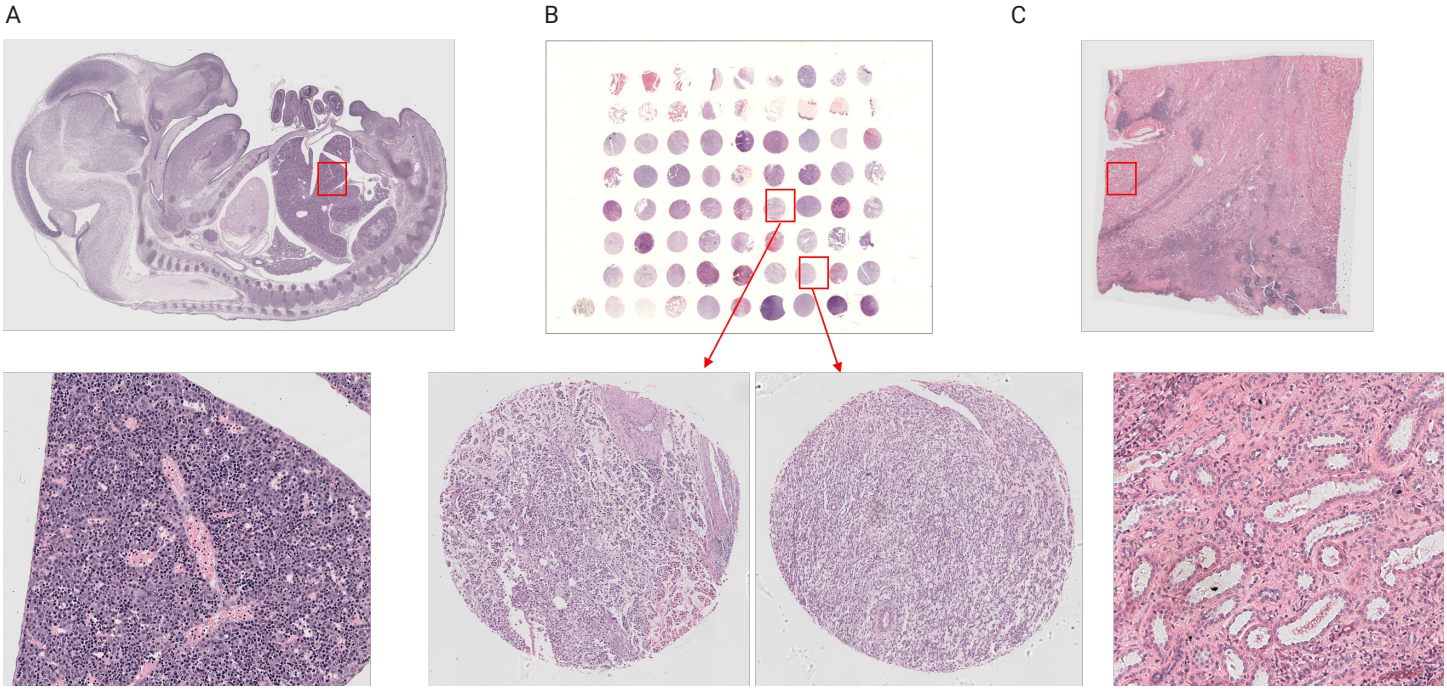

Figure 3. Color brightfield imaging of H&E tissue slides. (A) Mouse embryo precision calibration tool for automatic stage alignment and illumination correction. (B) Human organ tissue microarray. Correction is automatically applied during montage acquisition to eliminate tiling artifacts in fluorescence and color brightfield imaging modes. (C) High-resolution Olympus UPLXAPO 20x 0.8 NA and UPLXAPO 40x 0.95 NA objectives with wide field of view camera. (D) Agilent BioTek Cytation 9 slide capacity is scalable to 120 microscope slides with compatible Agilent BioTek BioStack microplate stacker.

ROI-based workflow and slide capacity

For slide-mounted tissue workflows, practical efficiency depends not only on slide capacity, but also on whether acquisition can be focused on meaningful sample regions. Cytation 9 uses a region-of-interest workflow to capture low-magnification overview images before high-magnification acquisition (Figure 4). Tissue regions can then be manually curated or automatically detected using Gen5 AutoROI module. This allows empty regions to be skipped, reducing acquisition time and storage requirements while preserving user control over final imaged regions.

Slide capacity can be extended by integrating Cytation 9 with an Agilent BioTek BioStack microplate stacker. Using a four-slide holder, capacity can reach up to 120 slides with 30-plate stacks. For batch workflows, overview images are collected first, ROIs are identified or modified, and the full batch can then be approved for high-magnification imaging of selected tissue regions. This approach supports routine batch imaging while keeping acquisition focused on tissue regions selected by the user or detected by the software.

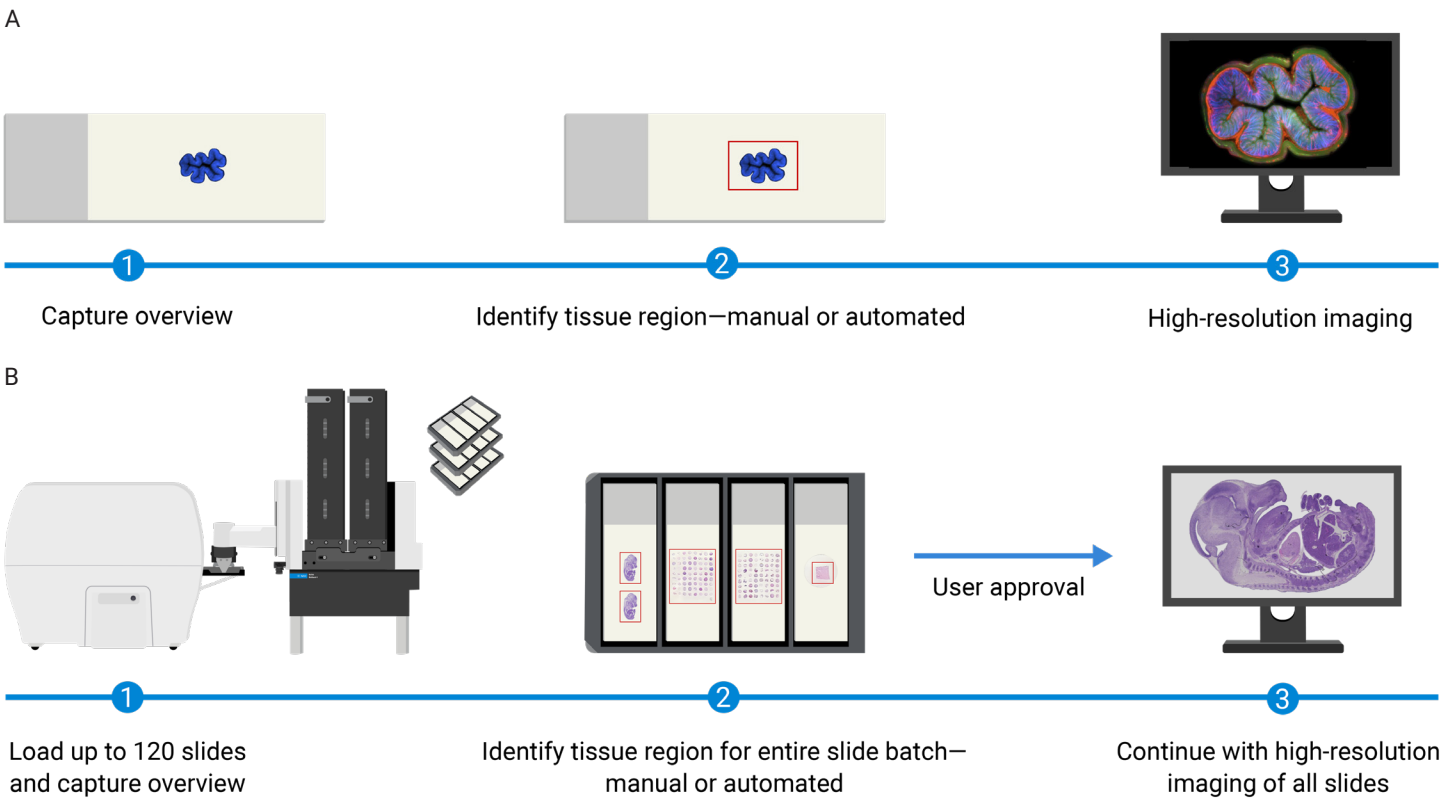

Figure 4. (A) Slide imaging workflow. Overviews of individual slide areas are captured automatically. Following manual or automatic tissue identification, high-resolution multichannel imaging proceeds for all identified regions. (B) High-capacity imaging workflow. The Agilent BioTek BioStack microplate stacker accommodates up to 120 slides. Overview images are captured for all slides before tissue regions are identified. Following user approval, high-resolution imaging proceeds for all slides and all regions (n = 96).

Conclusion

The Agilent BioTek Cytation 9 cell imaging multimode reader generated high-resolution fluorescence and color brightfield images from multiple slide-mounted tissue sample types, including H&E-stained and fluorescent tissue sections, as well as a tissue microarray. Automated calibration, laser autofocus, high-numerical-aperture objectives, and ROI-based acquisition supported consistent imaging while reducing unnecessary acquisition of empty slide regions. These capabilities allow slide-mounted tissue imaging to be incorporated into broader research workflows that include live- and fixed-cell imaging, as well as microplate-based imaging.

Products used in this application

Agilent products

BioTek Cytation 9 Cell Imaging Multimode Reader

www.agilent.com/lifesciences/biotek

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