

Applied robotics for enhanced throughput options in microscopy as demonstrated by automated Tissue Microarray (TMA) imaging



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Introduction

Historically, microscopy has been a hands-on method that can limit higher throughput options in imaging analysis. More recently, options for data analysis in microscopy have expanded from both the introduction of automated microscopy slide imagers, and the adaptation of microscopy slide techniques to imaging in microplates or other high throughput formats. An example of one of these adaptations is the tissue microarray (TMA), a technique where hundreds of individual tissue cores as small as 0.6mm in diameter and 2-5µm thick can be arrayed on a single microscopy slide allowing increased efficiencies in a number of common histology procedures. Already a high throughput solution, TMAs (Figure 1) have proven useful in many applications such as bio banking and archiving of biopsy and other tissue samples, disease diagnosis, classification and grading, quality control, antibody and staining optimization during assay development, and for meeting criteria such as the 'Validation and Verification of Immune Reactivity of All Classes of IHC's with a Panel of Normal Tissues or Cells' required by the U.S. FDA regulatory document *Guidance for Submission of Immunohistochemistry Applications* to the FDA; Final Guidance for Industry^{1,2}.

TMAs were chosen as a model for demonstrating montage and stitching image optimization at high throughput due to their unique geometry and mounting technique. Following microtome sectioning, paraffinized TMA slices are placed in water and the microscopy slide is dipped under the sheet and lifted up out of the water bath to capture the array. Although there is a window of time when the TMA can be repositioned on the slide, the result of this technique is that even panels of the same number and size of cores may not be mounted with the same center offset on a microscopy slide (Figure 1). Additionally, some TMAs may or may not have marker cores that can be offset from the remaining cores. Further, although BioTek microscopy slide adapters used here have been designed for standard 25 x 75 x 1mm microscopy slides, some tolerance should be included when calibrating x-y offsets for imaging to compensate for possible slide movement during robotic transfers and/or image carrier positioning.

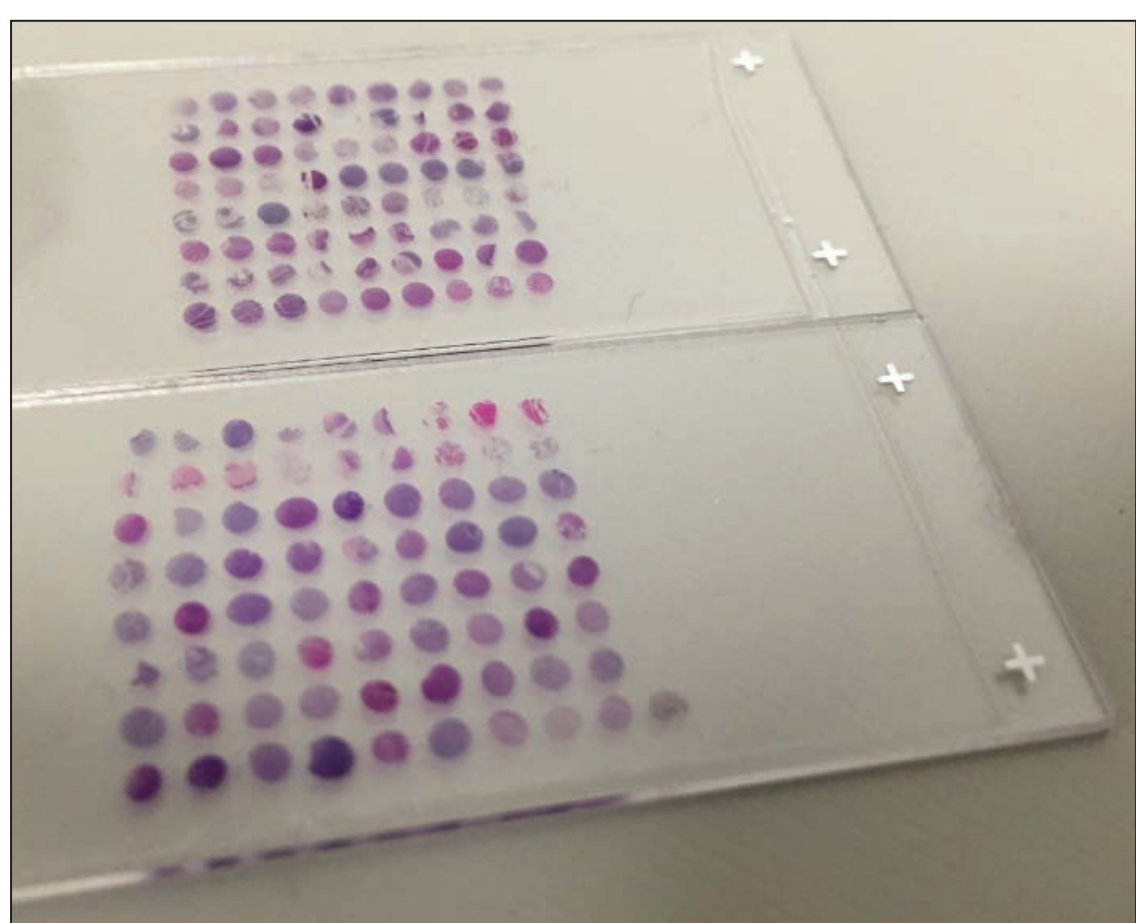


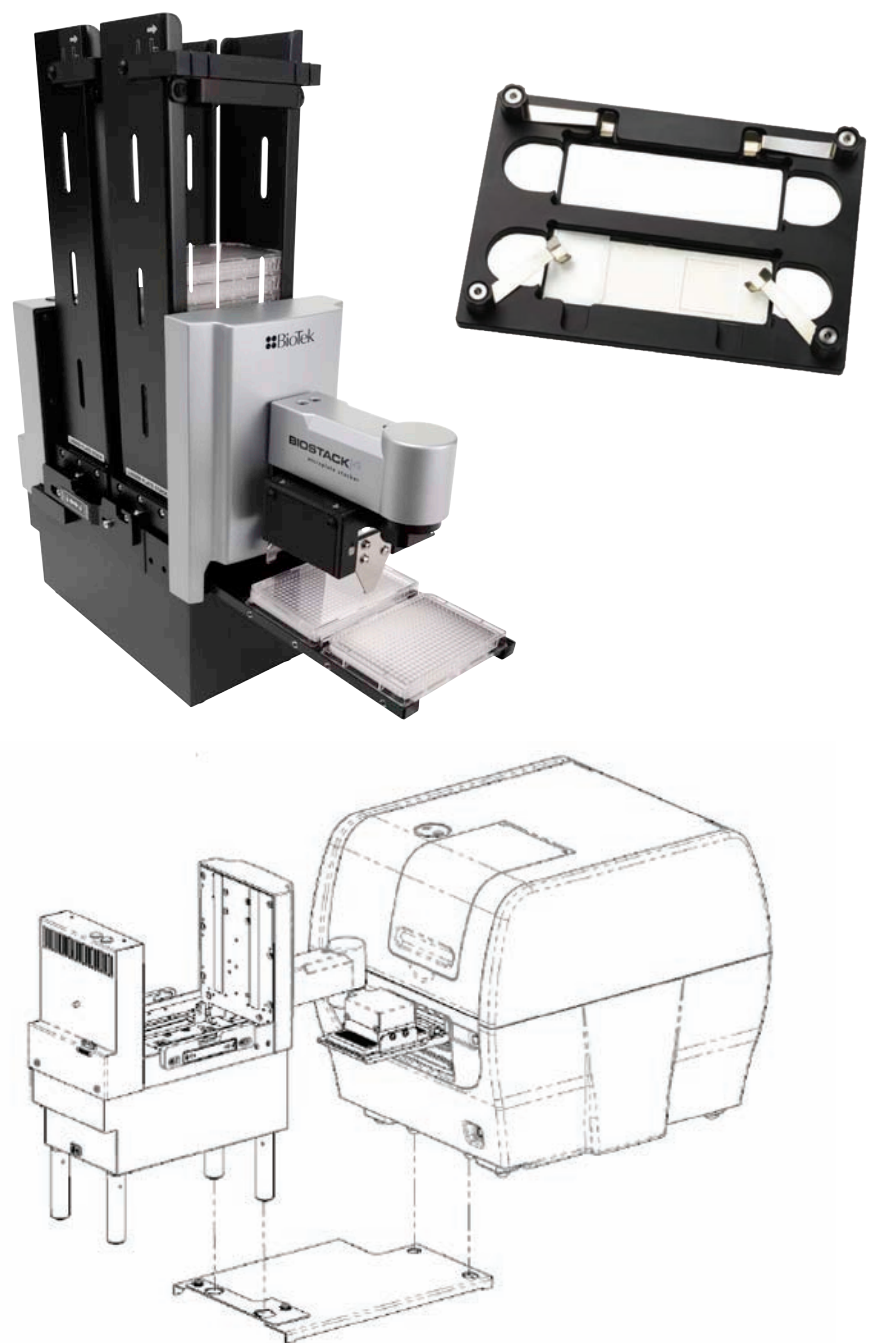
Figure 1. Two 9 x 8 tissue microarrays (TMAs) shown with different offset positions on the microscopy slide. The slide on the bottom includes a marker core, often embedded into arrays as a control and to assist with identifying panel locations, but also adding to asymmetric positioning between arrays. Customized x-y offsets and montage and stitching options available in BioTek's Gen5™ software can be used to compensate for these geometric anomalies during imaging on a Cytation™ 5, allowing mixed size TMAs to be run together in higher throughput via the BioStack™ 4 microplate stacker.

BioTek® Instrumentation



Cytation 5™ Cell Imaging Multi-Mode Reader is a uniquely integrated, configurable system that combines automated digital widefield microscopy with conventional multi-mode microplate detection. This instrument replaces multiple modules and software interfaces, yet is simple to setup and operate. With up to 60X magnification, and up to 6 onboard objectives, the microscopy module provides high-quality cellular and sub-cellular imaging in fluorescence, brightfield, color brightfield, and phase contrast channels.

Gen5™ v2.07 Software drives robotics control, and image and data capture and analysis. For the applications described here color brightfield and fluorescence imaging channels were used at 4X, 20X, and 40X either individually or in parallel. Both single imaging and montage and stitching of multiple images were utilized via features available in Gen5 as described in the method section.



BioStack™ 4 Microplate Stacker is a compact and versatile microplate stacker compatible with BioTek's washers, dispensers, detectors and imaging systems. The unique carrier design of BioStack brings unprecedented transfer speeds to increase throughput and enhance productivity. The BioStack 4 is compatible with a microscopy slide adapter shown left (BioTek p/n 1220548). Hands-free walkaway batch microscopy slide imaging is easily accomplished partnered with Cytation 5. BioStack models are available with 10, 30, or 50 carrier storage stacks, all removable and interchangeable to accommodate individual throughput needs. The 30 carrier stack was used for the applications presented here

Figure 2. The Cytation 5 imager and BioStack 4 are interfaced using an Integration Kit (BioTek p/n 7310053) as shown.

Materials and Methods

Materials

- 1 set¹ FDA Human Normal Organ Tissue¹ Microarray Panels and Core Specification Sheets, US Biomax, Inc. (p/n DA802, H&E stained) http://www.biomax.us/tissue-arrays/Multiple_Organ/FDA802 • Qty 6 'Top 4 Types of Cancer Test Tissue' Microarray Panels and Core Specification Sheet, US Biomax, Inc. (p/n TP242. H&E stained), http://www.biomax.us/tissue-arrays/Multiple_Organ/TP242 • Thyroid cancer and Adenoma tissue array (including TNM and clinical stage), US Biomax, Inc. (p/n TH641, unstained) • BioTek Slide Adapters p/n 1220548 • BioTek Integration Kit for Cytation 5 – BioStack 4 Interface p/n 7310053

Method

Optimization of image acquisition and high throughput automation of TMAs

An H&E stained 72 core TMA was placed into a slide adapter and x,y offsets and montage parameters were configured using manual mode within the protocol definition interface of Gen5 software at 4X magnification in color brightfield (Figure 3). A second 72 core TMA was placed on the holder, and both slides were imaged. X/y offsets were then reconfigured for the second slide. Two read steps were programmed into the protocol so that both TMAs could be imaged at two different geometric configurations. Then, six H&E stained 1.5 mm 24 core TMA slides were loaded into 3 additional BioTek microscopy slide holders, and all 4 holders were stacked vertically within the BioStack 4 supply tower. Gen5 software was programmed to sequentially load each slide holder from the microplate stacker to the Cytation 5 imager, returning the slide adapter to the stacker receiving tower following each imaging session. TMAs and individual cores were imaged using the defined protocol. Following the run, images were processed using a linear blend montage method on the red channel and a downsized image size of 7.35%. The same process was used to image select cores on 3 TMAs at 20X and 40X. For comparative purposes, a DAPI stained discarded prototype TMA donated by an area histology laboratory was also optimized using the same procedure at 4X using the DAPI fluorescent imaging channel, and is included to illustrate fluorescent imaging of a TMA. BioStack 4 was also used for that single slide transfer to and from the imager allowing hands-free walkaway imaging.

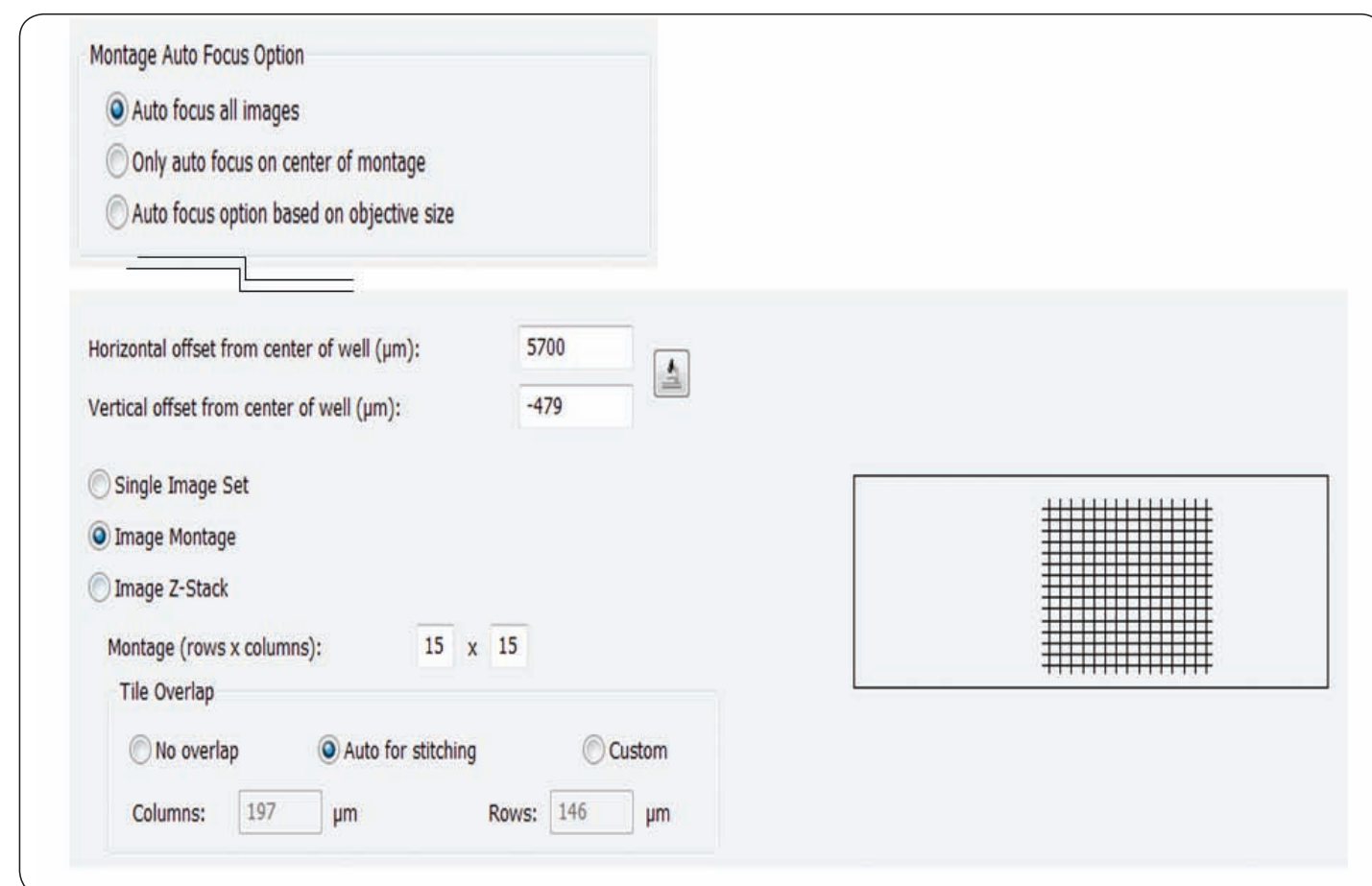


Figure 3. An example of the x-y offsets, focus setting, and montage and stitching options that were used to sequentially image a batch of 8 TMAs of 2 different panel sizes at 4X in color brightfield on a Cytation 5. Slides were transferred back and forth to the imager using the BioStack 4

Results

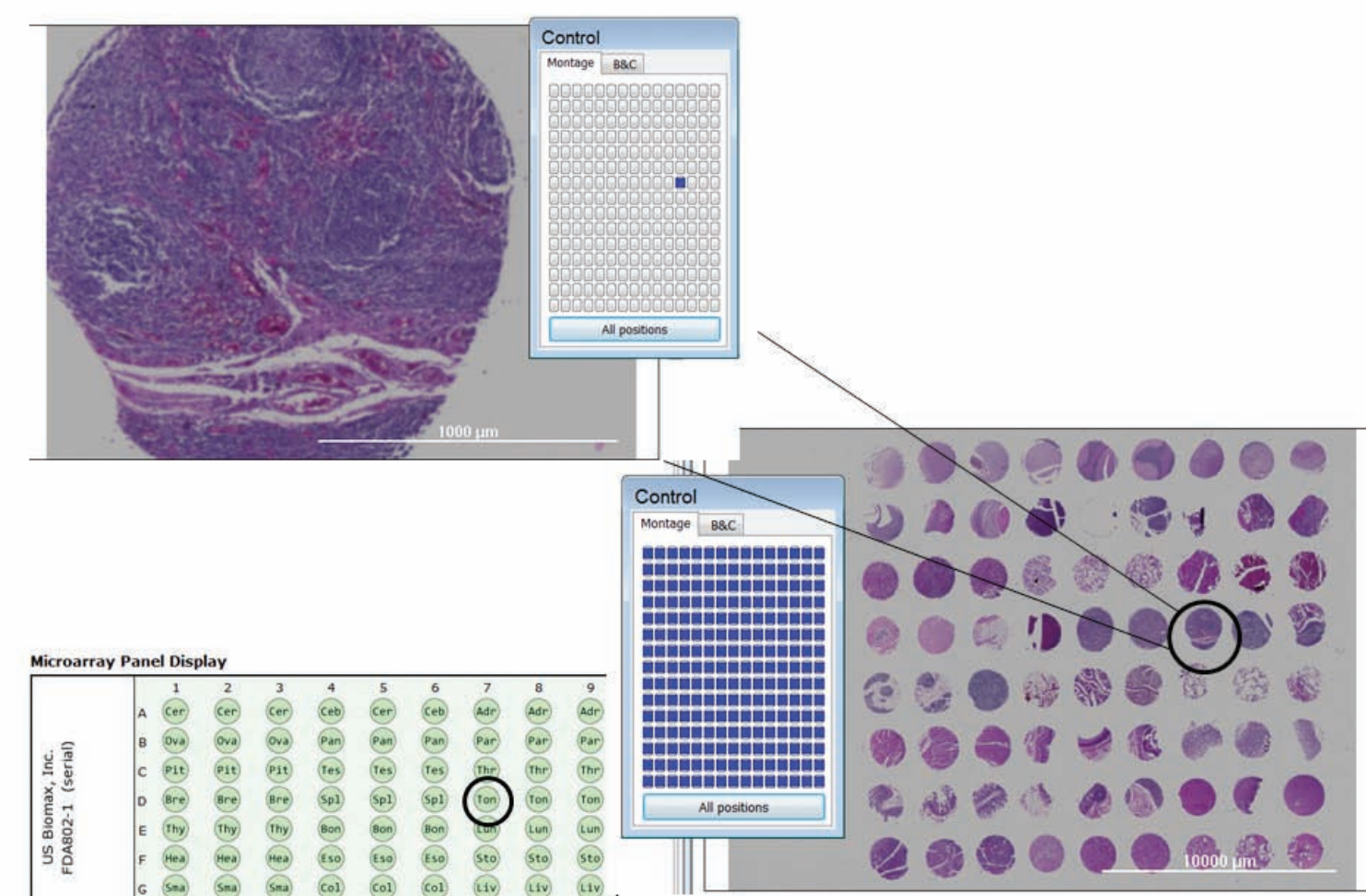


Figure 4. A 72 core tissue microarray imaged on Cytation 5 at 4X using montage and stitching options available in Gen5 software. This slide was run in a batch of 8 mixed sized TMAs using BioStack 4. The bottom right image shows the entire array displayed using the montage All Positions view. A core of normal tonsil tissue is shown in single tile view, selected by clicking on an individual tile in the Montage control panel, or double clicking directly on the montage image.

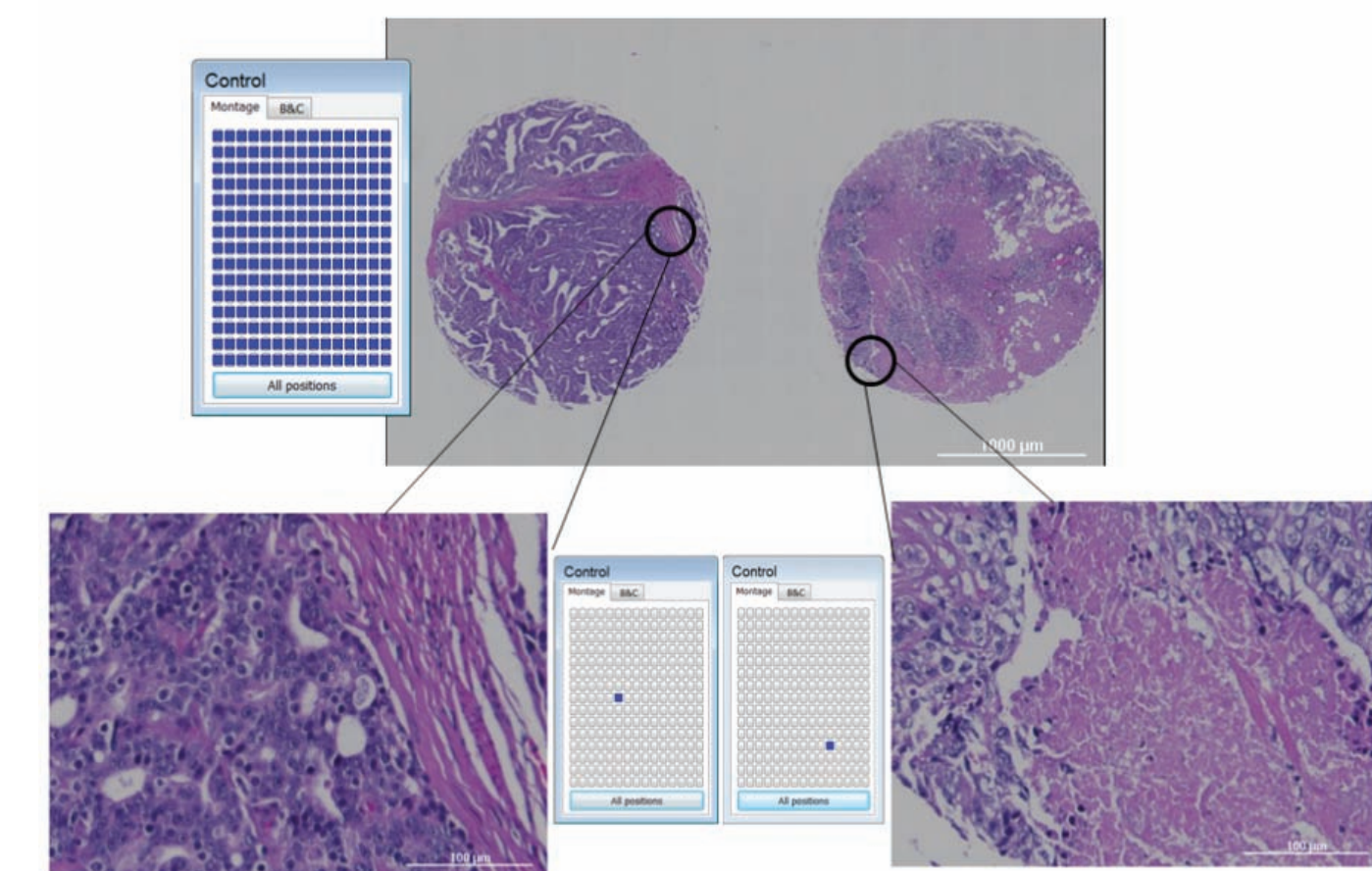


Figure 5. 15 x 15 montage @20X with area of detail for individual tiles shown. The left core is malignant prostate tumor, on the right is prostate adjacent tissue 1.5cm away from tumor. These cores are two different cases from a 24 core array.

Results Cont.

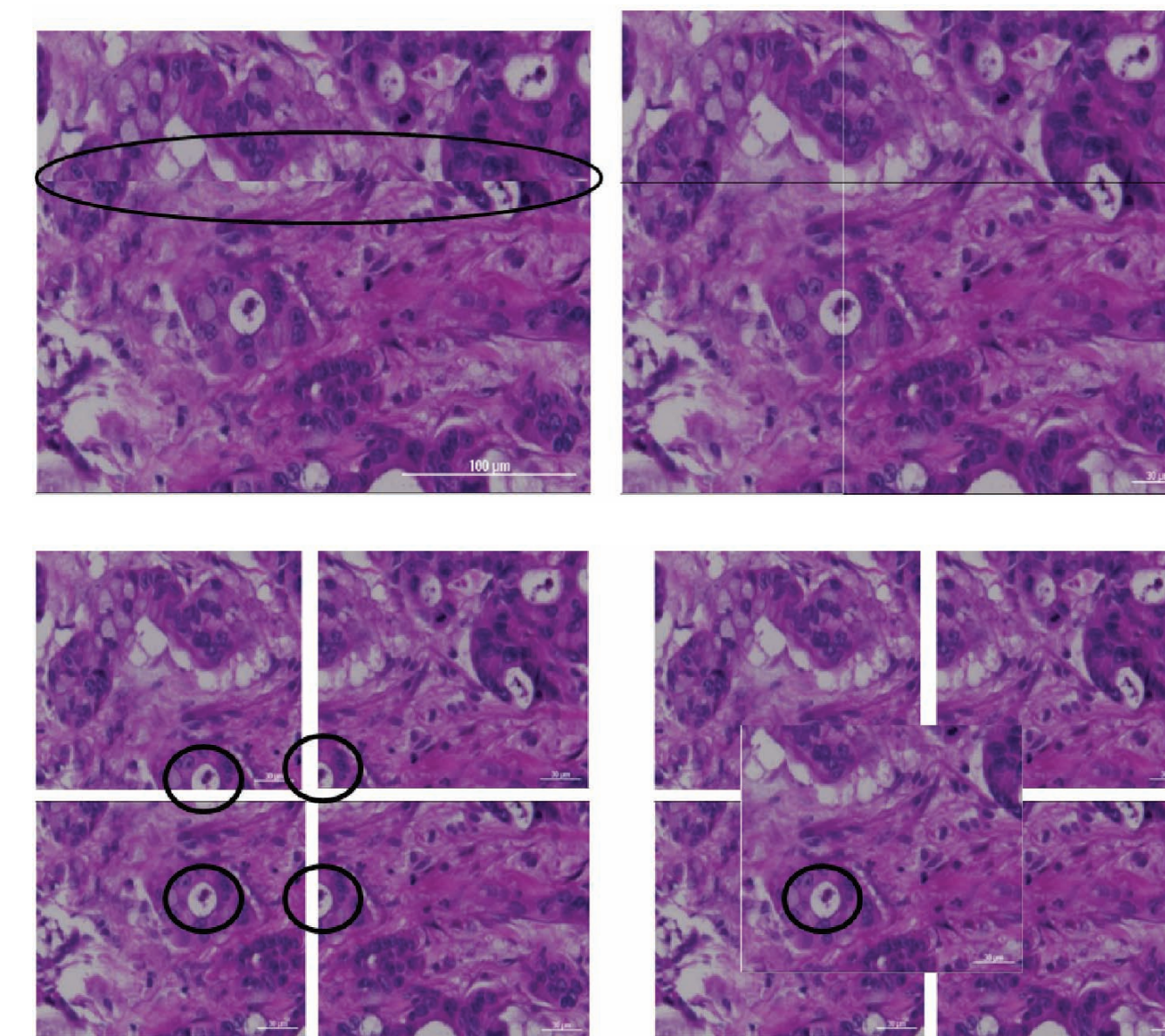


Figure 6. (Top left) default stitching overlap settings are asymmetric along the horizontal row (circled). Tile overlap values can be customized to correct this as shown top right by illustration of optimal 2x2 montage tile overlap for stitching @ 40X. (Bottom left) individual tiles of the 2x2 montage @ 40X. Circles show where each tile is imaged in relationship to the others using a distinguishable marker on the tissue. (Bottom right) overlay of a single image of the same core using the center offset defined for the 2x2 montage. The image was centered over the marker (circled) to illustrate the increased total surface area imaged by montage at the same magnification. All detail is from a 24 core array.

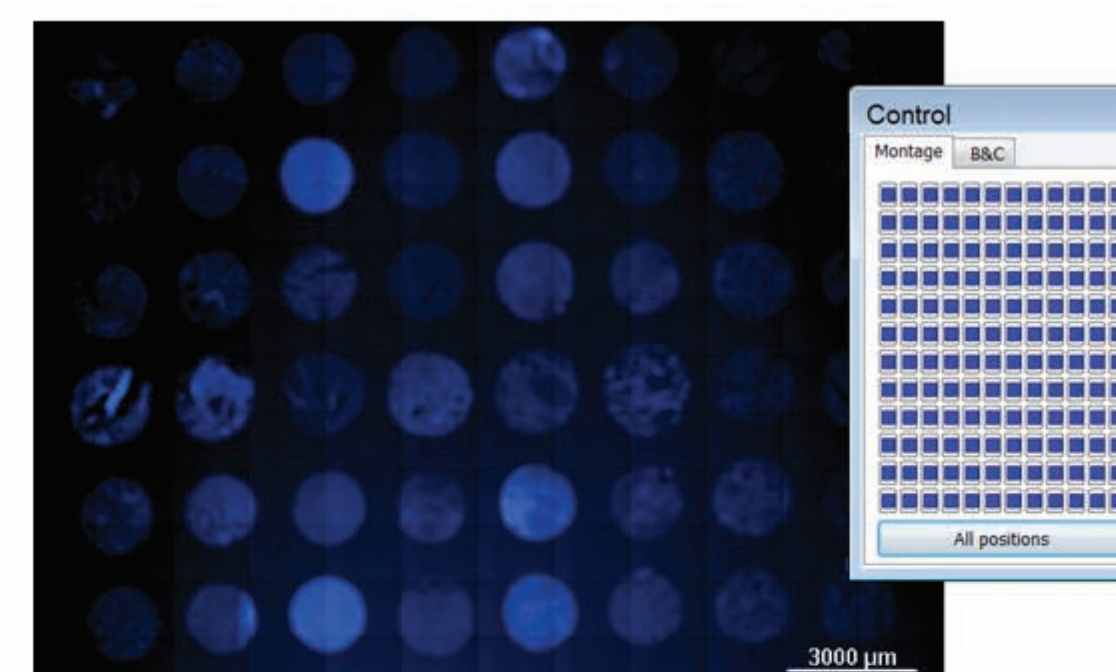


Figure 7. A discarded prototype TMA donated by an area histology laboratory stained with DAPI and imaged at 4X on Cytation 5 using montage and stitching in Gen5 software (top). Background haze is likely attributed to incomplete deparaffinization. Bottom left is a single image taken at 20X from the same array, and bottom right is H&E stained normal lung tissue @ 20X from another slice of the same array imaged by an independent source on another microscope for comparison (US Biomax p/n TH641).

Conclusions

- The built-in stitching and montage algorithms available in Gen5 v2.07 are ideally suited for imaging tissue microarrays in color brightfield and fluorescence modes at any throughput.
- Optimizing the montage size, tile overlap dimensions, x-y offsets and focus options enhances customized image acquisition useful for arrays of the same or different sizes to be run in a single batch.
- The montage control panel allows a view of either the entire stitched image or a close-up of each individual tile of the montage, allowing more detailed examination of stained tissue cores.
- The ability to include multiple read steps within a single protocol allows 'parallel montage' at multiple magnifications or geometric configurations, overcoming the challenge of imaging arrays of different sizes or with different x-y offsets at higher throughput, particularly when images of larger surface areas are desired at higher magnifications.
- At 20X, a 15 x 15 montage was found optimal for imaging pairs of 1.5 mm cores, offering enhanced detail via a larger field of view useful when imaging core pairs, such as malignant and adjacent tissue cores.
- At 40X a 2 x 2 montage provided 4 tiles that taken together covered a larger field of view than a single image at the same magnification on a portion of one 1.5 mm core sample. Custom tile offsets may be required to optimize tile overlap alignment if a final stitched image is desired from a 40X montage.
- Imaging at 4X using a 15 x 15 montage grid with auto focus on each image and custom x-y offsets configured to the largest array was found most favorable for capturing all cores of 72 and 24 1.5 mm TMAs.
- By means of any of the multi-mode imaging options available on the Cytation 5 the techniques shown here can be optimized on diverse microscopy slide applications using fixed human or animal cell lines, single tissue slices, partial or whole specimen mounts, or bacteria and yeast smears for example.