Application Note

Histology

Agilent

Trusted Answers

Detection and Automated Imaging of Regions of Interest (ROIs) when Performing Whole Slide Imaging (WSI)

Author

Peter J. Brescia, MSc
Agilent Technologies, Inc.

Abstract

In today's research and clinical environments, there is a movement towards whole slide imaging (WSI) as a means to transform physical specimens mounted on traditional microscopy slides or various sample vessels into a digital medium. Once digitized, the data can then easily be stored as a shared resource for a variety of purposes including pathology, diagnostics, and as scientific research and educational tools. The digital format is amenable to analysis using both traditional human methods as well as increasingly powerful computational algorithms. Augmented microscopy allows the development of streamlined methods to locate regions of interest (ROIs) on a slide, within a microplate well, or within a given sample. Once ROIs are selected, automated image acquisition is performed using a range of available imaging methods and subsequent automated image analysis, as required.

Introduction

Since the development of the first instruments to visualize microscopic objects by Antonie Van Leeuwenhoek in the 17th century, efforts have been focused on improved methods to capture and analyze those objects.[1] While the first images consisted of hand drawings, some of which were elaborately detailed, in the latter half of the 20th century, technological advancements allowed digital imaging and subsequent computerized methods of analysis.[2] Sample variety spans the diverse biological and physical universe including tissue acquired in clinical settings for diagnosis, samples in basic research initiatives across disciplines and organizations, and those suitable for educational and/or collaborative purposes.[3] Analyses include quantification of a range of observable parameters such as object counting and quantification of object size as well as more challenging metrics such as area, pixel intensity, and subpopulation analysis of objects in multichannel fluorescent images. Furthermore, digital images provide a means to standardize and automate analysis of some of the information present in a more precise, reproducible, and objective manner compared to traditional human analysis.[2]

Around the turn of the century the emerging focus on whole-slide imaging (WSI) of glass slides began to receive broader acceptance with improvements in rapid image acquisition and data management solutions.[4] Much of the driving force for this development was digitization of traditional histology slides that can be viewed via a computer monitor or mobile device for clinical pathology.[2] In particular, relieving the burden of routine image analysis of standard screening would significantly increase sample throughput.[3] However, the applications are broad for such technology, having merits for academic research, pharmaceutical drug discovery and development programs, as well as for biotechnology companies. The objective is to simplify the workflow by quickly identifying ROIs using a low-magnification optical path followed by image acquisition of the selected region at a higher magnification.

The ROI feature can be useful for scanning a slide containing one or more tissue sections in color brightfield (CBF), for example H&E-stained histological tissue sections or biopsy cores. The usefulness is extended by scanning fluorescently labeled tissue sections and imaging those ROIs using one or more channels at higher magnification such as for the assessment and quantification of biomarkers.[4] Additionally, more traditional imaging modes such as CBF, brightfield, and phase contrast can be combined with fluorescent channels resulting in rich data set. Provided in this application note are two examples of the use of a combination upright and inverted automated image acquisition system for performing WSI using ROIs where applicable.

Materials and methods

Instrumentation

The Agilent BioTek Cytation 7 cell imaging multimode reader combines automated digital upright and inverted widefield microscopy with conventional multimode microplate reading in a unique, patented design. The inverted microscopy module provides sample visualization from 1.25x to 60x magnification in fluorescence, brightfield, and color brightfield for a broad range of applications. The upright microscopy module with reflected light imaging enables even more applications such as ELISpot or fast slide scanning and ROI detection workflows. Cytation 7 includes continually variable bandpass

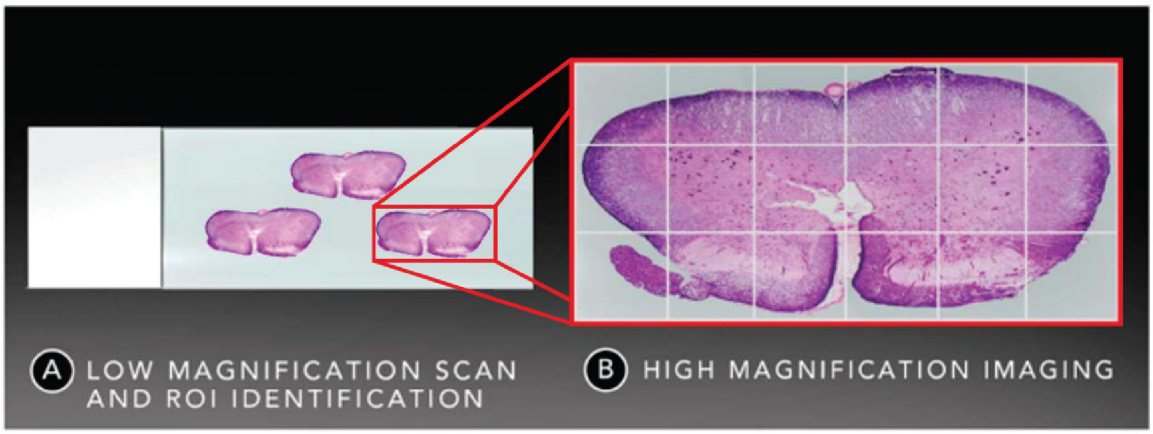

Figure 1. Whole-slide imaging (WSI). Upright camera and optics can quickly image a large number of sample mounted on a single glass slide at low magnification. Regions of interest (ROIs) are identified and subsequently imaged at higher magnification for analysis.

monochromators for versatility and performance for general multimode plate reader applications. Temperature control to 45 °C, an optional Peltier Cooling module, gas control, and shaking expand the applications for kinetic cell-based assays. Cytation 7 is controlled by Agilent BioTek Gen5 microplate reader and imager software, which combines ease-of-use with powerful processing and analysis capabilities.

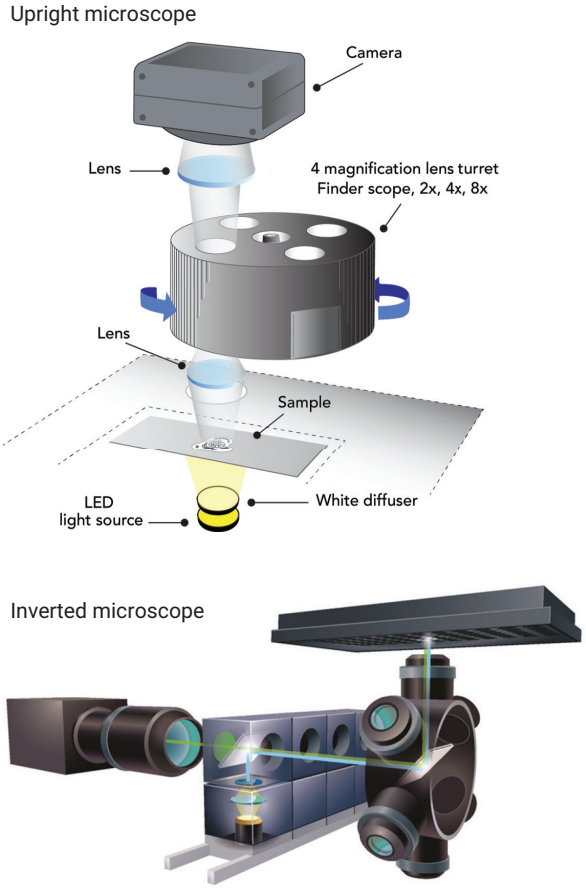

Figure 2. Agilent BioTek Cytation 7 cell imaging multimode reader. Upright and inverted microscopes provide wide range of magnifications (1.25x to 60x). The upright, low-magnification optical path is used for whole-slide imaging. The inverted microscope is then used to image regions of interest at higher magnification using a variety of imaging methods (e.g., phase contrast, epifluorescence, color brightfield, etc.).

Methods

Stomach tissue, H&E-stained (Carolina Biological Supply Company, Burlington, NC, USA). Human prostate tissue microarray (cancer), H&E-stained (part number NBP2-30169) (Novus Biologicals Europe, Abingdon, UK). WSI of the stomach tissue was performed at 2x magnification, 4 × 4 montage, using transmitted color brightfield through the upright digital microscope in the Agilent BioTek Cytation 7 to visualize and select ROIs. Three ROIs were selected and imaged at 10x magnification in color brightfield using the inverted WFOV digital microscope. WSI of the microarray was performed at 2x magnification, 3 × 5 montage, using transmitted color brightfield through the upright digital microscope to visualize and select ROIs. Fifty ROIs were selected and imaged at 10x magnification in color brightfield using the inverted WFOV microscope.

Results and discussion

Human prostate tissue microarray

A microarray of prostate tissue cores containing 40 adenocarcinoma samples, with histologic grades (Gleason scores) ranging from 6 to 10, and 9 matching normal tissue samples, from patients between the ages of 44 and 75 years old, were H&E stained for imaging. Sample cores were 2.0 mm in diameter with a section thickness of 4 µm arranged in a 5 × 10 matrix. Initial WSI was performed using the parameters listed in Table 1 generating a single large, low-resolution image of an area slightly larger than that containing the tissue cores. ROIs were selected for each core to minimize imaging of the background area. The ROIs were subsequently imaged at 10x magnification using the parameters listed in Table 1, resulting in 50 unique sets of tiled images (3 × 3 montage) for each sample core; 49 prostate tissue samples and one carbon location marker. The images were processed into a single image using automated image stitching resulting in 50 individual images for future analysis and archiving (Figure 3).

Table 1. Agilent BioTek Cytation 7 imaging settings.

Microscope	Image Mode	Image Method	Image Processing	Magnification	Automated Functions
Upright	Color brightfield	Montage	Stitching	2x	Focus, exposure, LED intensity
Inverted	Color brightfield	Montage, Auto based on ROI	Stitching	10x, WFOV	Focus, exposure, LED intensity

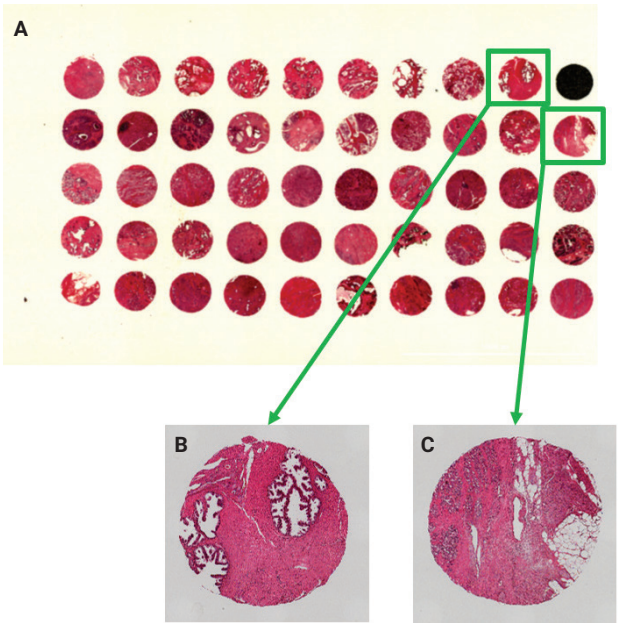

Figure 3. Human prostate tissue microarray. (A) Low-resolution image of microarray, (B) representative image of a 3 × 3 stitched montage of a prostate adenocarcinoma using a WFOV camera at 10x magnification and (C) representative image of normal matched prostate tissue as described in the Methods section.

Mammalian stomach tissue: cardiac, fundic, and pyloric regions

Three sample tissue sections representing the cardiac, fundic, and pyloric regions of the stomach with a section thickness of ~4 µm arranged under a 18 mm, circular coverslip were imaged. Initial WSI was performed using the parameters listed in Table 1 generating a single large, low-resolution image of an area slightly larger than the coverslip. ROIs for each tissue section were selected to minimize background area and imaged at higher resolution using the parameters listed in Table 1 resulting in three unique sets of tiled images (3 × 6, 2 × 4, and 3 × 7 montages) for cardiac, fundic, and pyloric regions, respectively. Tiled images were stitched into single images using automated image stitching, resulting in three individual images for future analysis and archiving (Figure 4).

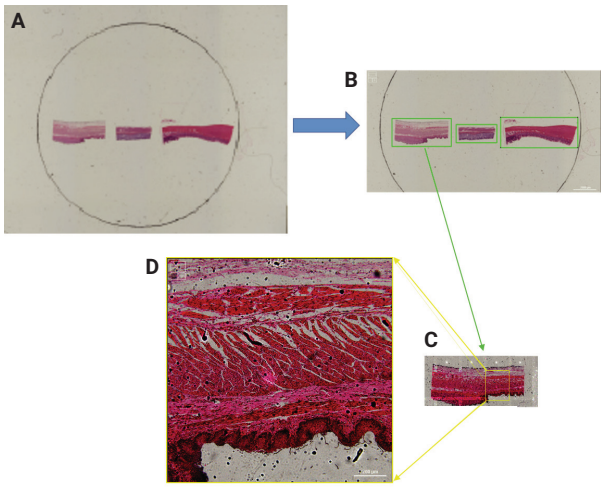

Figure 4. Mammalian stomach: cardiac, fundic, and pyloric regions. (A) Low-resolution image of tissue samples, (B) selection of ROIs, (C) stitched image of ROI at 10x magnification, and (D) zoomed area representative of the high-resolution stitched image.

Conclusion

The ability to capture images digitally has led to a paradigm shift resulting in simplified workflows and sharing for diagnostics, education, and basic scientific research. Automated imaging solutions have further expanded the capabilities by offering rapid whole-slide imaging (WSI) or other various vessel types to determine regions of interest (ROIs) for subsequent higher-resolution imaging and analysis. Additionally, the inclusion of multiple imaging modes such as color brightfield, phase constrast, and epifluorescence microscopy greatly expands the breadth of possible applications. Two examples shown in this application note – imaging of prostate tissue cores and stomach tissue sections in color brightfield – show the ability to rapidly image entire vessel surfaces at low resolution, select only those regions of interest, and capture high-resolution images of the ROIs using automated imaging methods.

This paradigm results in a powerful system, that when coupled with image-based computational analysis tools, provides a viable solution to increase sample throughput.

References

1. Meijer, G. A. *et al.* Origins of… Image Analysis in Clinical Pathology. *J. Clin. Pathol.* **1997**, *50*, 365–70.
2. Aeffner, F. *et al.* Introduction to Digital Image Analysis in Whole-slide Imaging: A White Paper from the Digital Pathology Association. *Journal of Pathology Informatics* **2019**, *10*. doi: 10.4103/jpi.jpi_82_18.
3. Gurcan, M. N. *et al.* Histopathological Image Analysis: a Review. *IEEE Reviews in Biomedical Engineering* **2009**, *2*, 147–171. doi: 10.1109/RBME.2009.2034865.
4. Zarella, Mark D. *et al.* A Practical Guide to Whole Slide Imaging. A White Paper From the Digital Pathology Association. *Arch. Pathol. Lab. Med.* **2019**, *143*, 222–234.

www.agilent.com/lifesciences/biotek

RA44412.5419560185

Printed in the USA, May 4, 2022
5994-3404EN

Agilent
Trusted Answers