TECHNICAL NOTE

Improved high-content imaging of tissue sections.

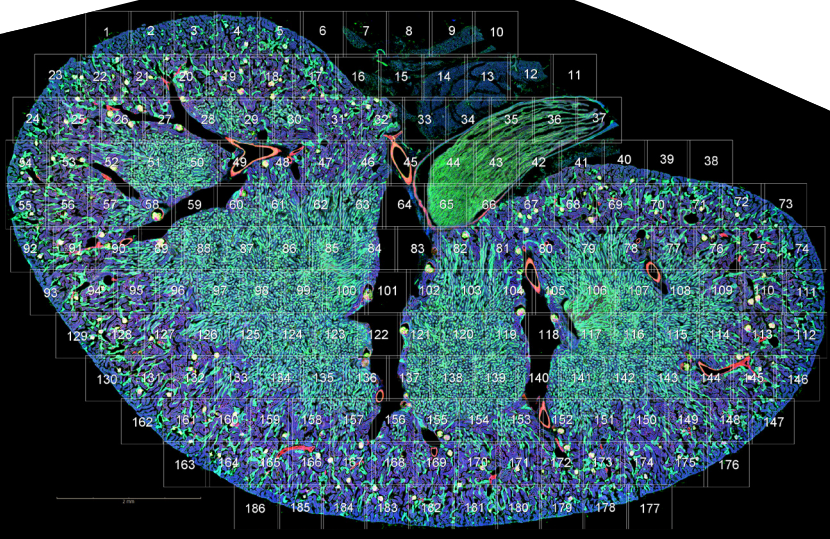
Learn how to:

- Detect the location of tissue sections in X, Y and Z dimensions
- Decrease image acquisition time 4-fold
- Decrease data volume 3-fold

Histological tissue sections are a key sample type to bridge the gap between cell culture-derived data and the *in vivo* situation and play a crucial role in understanding molecular alteration in disease.[1] However, tissue sections are often considered difficult samples for automated image acquisition and analysis and their use in screening approaches can be challenging. For example:

- The position of the tissue section varies in x, y and z between slides.
- The tissue section may not be completely flat, hence the offset between coverslip and section varies.
- The coverslip may not be mounted completely parallel to the slide introducing a tilt.

These factors can have a significant impact on the feasibility and speed of high-resolution imaging. Therefore, it would be of great advantage if the exact positions of the tissue sections on the slides could be identified prior to high resolution, high magnification image acquisition to avoid generating out-of-focus images and unnecessary data. Part of Harmony® high-content analysis software, PreciScan™ provides intelligent image acquisition capabilities. Here we demonstrate how PreciScan can be applied to slide imaging to identify the x, y and z positions of tissue sections and how this speeds up measurement time and minimizes data volume.



3D PreciScan of a tissue section - working principle

The PreciScan workflow enables the user to image only the objects of interest in an automated manner. PreciScan image acquisition consists of three sequential steps (Figure 1). The first step is a pre-scan of the sample using a low magnification objective. In the second step, the pre-scan images are automatically analyzed using an online analysis to determine the XYZ positions of the desired objects. In the third and final step, the identified positions are used to set image fields, capturing the objects in a re-scan with a higher magnification objective. As an example of a standard tissue section, we used a FluoCells™ slide (Invitrogen #F24630) containing a mouse kidney section stained with DAPI, Alexa Fluor® 488 wheat germ agglutinin and Alexa Fluor® 568 phalloidin.

The PreciScan workflow was also compared with a conventional scan using a laser-based autofocus on the Opera Phenix® high-content screening system. A z-stack of 15 µm was sufficient to cover the whole mouse kidney tissue section. Without PreciScan, an additional 20 µm needed to be added due to the 20 µm height difference throughout the tissue section. At a step size of 1 µm, this translates to 36 planes for the whole z-stack. Furthermore, a large area needs to be imaged due to the lack of x/y positional information. Thus, for a conventional scan with the 20x objective, about 336 fields needed to be acquired, each with 36 planes and 3 channels, equating to ~45 GB of data and an acquisition time of ~74 min. In contrast, with PreciScan, using a 10x objective for the pre-scan and a 20x water immersion objective for the re-scan, the total data generated was ~15 GB, with an acquisition time of ~19 min. (Table 1). The use of PreciScan resulted in a 3-fold decrease in data volume and 4-fold decrease in acquisition time.

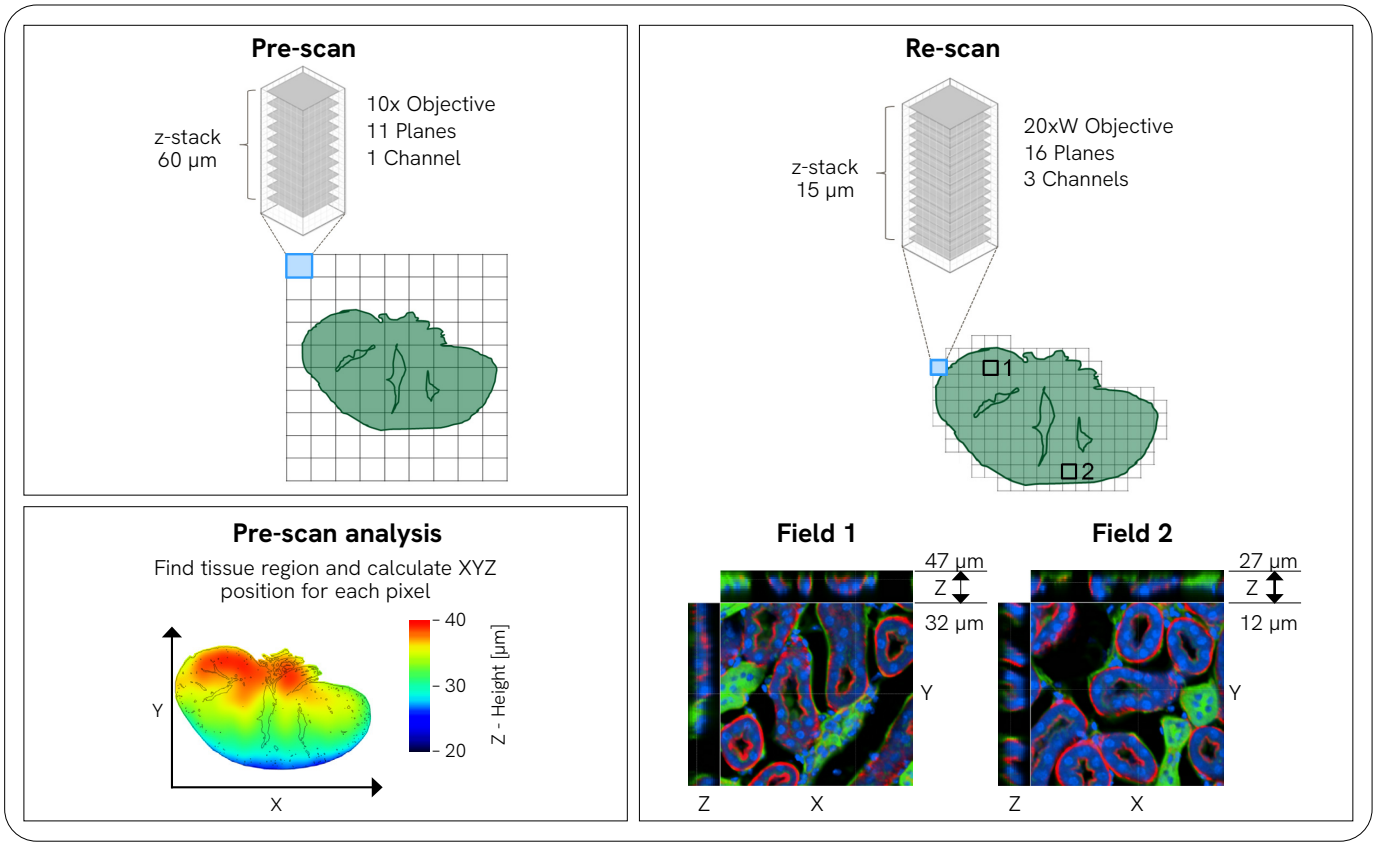


Figure 1. 3D PreciScan intelligent image acquisition enables the identification of the x, y and z positions of tissue sections on slides. The automated procedure consists of three sequential steps. First a low magnification pre-scan of the tissue section slide is performed, generating a one channel z-stack which covers the entire section in XYZ. An online analysis of the pre-scan precisely detects the tissue region and calculates the exact position of the tissue section. The color gradient within the analyzed tissue region indicates a z-position variation of 20 µm throughout the section. In the final step, the identified x, y and z coordinates are used to set image fields to capture only the tissue section at different z-heights in a re-scan measurement using the 20 x W objective. As a result, in the example, the absolute z values of the 15 µm z-stack changed according to the varying z-position of the tissue section. During the re-scan, example Field 1 was imaged with a z-stack ranging from 32 µm to 47 µm and Field 2 from 12 µm to 27 µm. The tissue section is a FluoCells® slide (Invitrogen #F24630) containing a 10 µm cryostat section of mouse kidney stained with Alexa Fluor® 488 wheat germ agglutinin (green), Alexa Fluor® 568 phalloidin (red) and DAPI (blue).

Table 1. 3D PreciScan decreases data volume by a factor of 3 and measurement duration by a factor of 4. As the precise x/y position of the tissue section on the slide as well as the suitable z-height for imaging is not known, a conventional automated scan without object-based imaging needs to cover a large area and z-volume to ensure imaging of the whole tissue section. This generates about 44.8 GB of data and requires about 74 minutes acquisition time on the Opera Phenix system. PreciScan allows the x/y scan area and z-volume to be decreased significantly, resulting in three times less data and a 4-fold decrease in acquisition time.

	Conventional scan	3D PreciScan	
Experimental setup	Scan (20xW): 336 fields 36 planes 3 channels (simultaneous)	Pre-scan (10x): 70 fields 11 planes 1 channel	Re-scan (20xW): 182 fields 16 planes 3 channels (simultaneous)
Data volume per tissue section	44.8 GB	1.8 GB	12.7 GB
Image acquisition time per tissue section	73.6 min	18.2 min	

Conclusion

Automated image acquisition and analysis of tissue sections can be challenging due to the inherent height variations throughout the specimens. Here we show that PreciScan intelligent image acquisition compensates for height variations throughout tissue sections by identifying the x, y and z- position of the tissue section on the slide, thereby enabling a significant reduction in data volume and acquisition time.

Reference

1. Liu M, Ylanko J, Weekman E, Beckett T, Andrews D, McLaurin J. (2019) Utilizing supervised machine learning to identify microglia and astrocytes in situ: implications for large-scale image analysis and quantification *J Neurosci* Methods. 2019 Sep 5;328:108424. doi: 10.1016/j.jneumeth.2019.108424.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
(800) 762-4000
www.revvity.com