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Authors: Giorgio Ioannou; Sacha Gnjatic, PhD		SOP Number: HIMC-5048	
Approvals			
HIMC	Quality Assurance		Revision: 0
Seunghee Kim-Schulze, PhD	Kai Nie		Effective Date: 06/06/2024
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1. Purpose

The purpose of this document is to outline a standardized procedure on how to scan and store stained histology slides using the Aperio AT2 DX Slide Scanner from Leica Biosystems.

2. Abbreviations

MICSSS: Multiplexed Immunohistochemical Consecutive Staining on a Single Slide
 QC: Quality Control

3. Safety Considerations / Personal Protective Equipment

Paraffin sections on glass slides are not considered to be biohazards and do not require personal protective equipment during handling, whereas, operators may opt to use personal protective equipment for handling glass slide and paraffin section on top of it.

4. Materials and Equipment


- 4.1. Stained and coverslipped slides
- 4.2. Aperio AT2 DX Slide Scanner and a connected desktop workstation

5. Quality Control


Please see section 6.4 and 6.5 for details.

6. Method

- 6.1. Start Windows operating system desktop computer connected to the scanner.
- 6.2. Click “Aperio ScanScope Console” icon to start the scanning software.
- 6.3. There are two scanning option modes: Single Scanning Mode is for individual slides and Batch Scanning Mode is for multiple slides.
 - 6.3.1. **Single Scanning Mode:**
 - 6.3.1.1. Place the slide face up on the slide tray with the label pointed toward the front.
 - 6.3.1.2. Click the Eject button underneath “Stage” on the bottom left corner of the console, set the slide name, and set the resolution to either 20x or 40x. 20x resolution is mostly preferred because of its optimal image size advantage. Click the Snapshot button.

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- 6.3.1.3. Once the snapshot has been taken click the Auto Select Tissue button to automatically select the entire tissue. Alternatively, the area to be scanned can be adjusted manually by clicking and dragging the corners of the scanning selection area in green.
- 6.3.1.4. Click on the Auto Select Focus Points button to automatically add focus points across the area of the tissue. Alternatively focus points can be manually added by double clicking on the desired areas.
- 6.3.1.5. Click and drag the Calibration Point to place it on a blank area of the slide that is coverslipped and that contains no tissue.
- 6.3.1.6. When everything is ready click the Scan button to start scanning.
- 6.3.2. **Batch Scanning Mode:**
- 6.3.2.1. Place the slides into the slide rack in the orientation depicted on the label on the rack.
- 6.3.2.2. Click the Eject button on the console located under the rack that you would like to use in the slide rack carousel.
- 6.3.2.3. Open the door to the slide rack carousel by swinging it to the left by using the door handle.
- 6.3.2.4. Insert the slide rack along the guide rail. When the rack is inserted properly, a slide rack schematic will appear on the console in the corresponding rack that the slide rack was inserted.
- 6.3.2.5. Close the door to the slide rack carousel by swinging it to the right by using the door handle.
- 6.3.2.6. Press and hold the Control key on the keyboard while clicking on all the positions in the rack that contain a slide on the console.
- 6.3.2.7. Click the Snapshot button. The scanner will start taking snapshots of all the selected slides placed in the rack.
- 6.3.2.8. After the snapshots have been taken, click on the View Slide button on the console next to the first slide in the rack. Set the name of the slide.
- 6.3.2.9. Click the Auto Select Tissue button to automatically select the entire tissue. Alternatively, the area to be scanned can be adjusted manually by clicking and dragging the corners of the scanning selection area in green.

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- 6.3.2.10. Click on the Auto Select Focus Points button to automatically add focus points across the area of the tissue. Alternatively, focus points can be manually added by double clicking on the desired areas.
- 6.3.2.11. Click and drag the Calibration Point to place it on a blank area of the slide that is coverslipped and that contains no tissue. Click the Back button.
- 6.3.2.12. Repeat sections 6.3.2.8-6.3.2.11 for the rest of the slides.
- 6.3.2.13. Press and hold the Control key on the keyboard while clicking on all the positions in the rack that contain a slide on the console. Set the resolution to either 20x or 40x. 20x resolution is mostly preferred because of its optimal image size advantage.
- 6.3.2.14. When everything is ready click the Scan button to start scanning. The scanner will start scanning all the selected slides in the slide rack.
- 6.4. After scanning is completed, slide status will be shown for each slide scanned. Blue means the slide is in the queue to be scanned, yellow means that slide is currently being scanned, green means the scan was successful, and red means an error has occurred. Once generated the scans get automatically saved in a subfolder labeled with today's date in a folder labeled "Images" on the desktop of the computer connected to the scanner.
- 6.5. Images are reviewed for QC by double clicking on the image file. This will trigger the Aperio ImageScope software which is the image viewer software for the Aperio AT2 DX scans.
- 6.6. Scanned images are stored in a protected shared drive with proper organization.
 - 6.6.1. Image names should include at least biomarker position in the panel (for MICSSS projects), biomarker stained, case ID, and cross section number.

7. References

- 7.1. Aperio AT2 DX User Guide (Rev. B)