
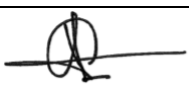
 <b>Mount Sinai</b>		<b>Icahn School of Medicine at Mount Sinai</b>	<b>Human Immune Monitoring Center</b>
<b>Hamamatsu NanoZoomer Slide Scanner</b>			<b>Page 1 of 4</b>
<b>Authors:</b> Guray Akturk, MD; Sacha Gnjatic, PhD			<b>SOP Number:</b> HIMC-...
<b>Approvals</b>			
<b>HIMC</b>		<b>Quality Assurance</b>	
Seunghee Kim-Schulze, PhD		Jose Lacunza	
			
			<b>Revision:</b> 0
			<b>Effective Date:</b> 2019-10-03
			<b>Supersedes Date:</b> New

### 1. Purpose

The purpose of this document is to outline a standardized procedure on how to scan and store stained histology slides using Hamamatsu NanoZoomer S60 Slide Scanner.

### 2. Abbreviations

LED: Light Emitting Diode

MICSSS: Multiplexed Immunohistochemical Consecutive Staining on a Single Slide

QC: Quality Control

ROI: Region of Interest

### 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. Hamamatsu NanoZoomer S60 and a connected desktop workstation

### 5. Quality Control

Please see section 6.8 and 6.9 for details.

### 6. Method

6.1. Start Windows operating system desktop computer connected to the scanner.



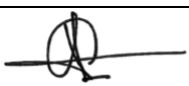
6.2. Click "NDP.scan" icon to start the scanning software

6.3. Open the slide cassette access door by sliding it to the left gently by using the door handle.

6.4. Place the slides into the cassette

6.5. Insert the slide cassette along the guide rail. When the cassette is inserted properly, the LED light which is located at the upper left of each cassette insertion section will light up white. If it is not inserted correctly, the LED will blink.

6.6. Close the slide cassette access door by sliding it the opposite side by using the door handle.

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6.7. After placing the cassettes into the NanoZoomer, two scanning options will appear on the main screen. Single scan mode is for individual slides and Batch Scan mode is for multiple slides.

#### 6.7.1. Single Slide Mode:

6.7.1.1. After closing the cassette access door and choosing Single Slide Mode, click Load Next Slide button

6.7.1.2. Set the slide name and path from the Filename section. Automatic option set it to a predetermined location. By clicking ‘...’, this predetermined location can be changed.

**Note:** When settings button is clicked, there is output location settings under General Settings menu. This menu allows to set a predefined default Path and filename format for all images (eg, yy-MM-dd HH.mm.ss).

6.7.1.3. Brightfield mode is selected as default in this screen, do not change this setting.

**Note:** Brightfield mode profile can be tailored from Profile settings according to the need of a project. Profile settings provides the control of scan area, focusing, dynamic focus, thresholds, coverslip filter, minimum size, split tissue, focus pieces, and excluded are features.

6.7.1.4. Single Layer is selected on Layers setting.

6.7.1.5. Resolution can be set to 20x or 40x, however 20x resolution is mostly preferred because of its optimal image size advantage.

6.7.1.6. Scan Area button offers the choice between Manual ROI and Automatic selection of the ROI. Automatic works decently in most of the cases.



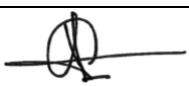
6.7.1.7. Focus points are set automatically inside the scan area, when Automatic selection is chosen for Scan Area setting. However, focus points can be added manually as well. If a focus point is desired to be deleted by the operator, it

can be done by moving the focus point outside the macro image by left mouse dragging or selecting delete all with the Focus button.

6.7.1.8. Focus points can be reviewed for thick or uneven tissues by enabling “Review Points”.

6.7.1.9. When everything is ready, click “Start Scan” button to start scanning process.

#### 6.7.2. Automatic Batch Mode

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- 6.7.2.1. Specify the name of each slide by entering Slide References setting. This screen shows the cassette scheme in the NanoZoomer. Slides names can be imported from an excel file. If slides have barcodes they are named automatically, however if operator wants to name them instead, NDP settings provides "Use barcode" or "Use slide reference" options (*see the note in 5.7.1.2*).
- 6.7.2.2. Batch type has two options including Fully Automatic and Semi-Automatic modes. Semi-Automatic mode offers the option of selecting ROI and placing focus points manually.
- 6.7.2.3. Brightfield mode is selected as default in this screen, do not change this setting (*see the note in 5.2.7.1.4*)
- 6.7.2.4. Single Layer is selected on Layers setting.
- 6.7.2.5. Resolution can be set to 20x or 40x, however 20x resolution is mostly preferred because of its optimal image size advantage.
- 6.7.2.6. When everything is ready, click "Start Batch" button to start scanning process. NanoZoomer will start scanning all slides placed in the slide cassettes. If Semi-Automatic mode is selected, Setup Scans button will be activated which allows selection of manual ROIs and focus points.
- 6.8. After scanning is completed, slide status will be shown for each slide scanned. Green tick means success, whereas red exclamation mark means an error has occurred.
- 6.9. Slides are reviewed by clicking View Scan buttons for QC. This will trigger NDP.View software which is the official image viewer for NanoZoomer images.
- 6.10. Scanned images are stored in a protected shared drive with proper organization.
  - 6.10.1. Under a specific named project folder, subfolders are created with marker names and images are saved under these folders.
  - 6.10.2. Image names should include at least case number, date, and order (for MICSSS projects).

## 7. References

- 7.1. NDP.scan Instruction Manual (Ver. 3.2/Rev.4)