Document Number: **SASoM/EQUIP/025.v3**

Title: **Image Acquisition using HistoRx® PM-2000, AQUA (Automated Quantitative Analysis)**

Version: **v3**

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Valid to:	01/07/2023

SOP History		
Number	Date	Reason for Change
v1	01/01/1013	Original
V2	02/07/2013	Minor Amendments
V3	02/07/2018	Update

1.0 Purpose –
The purpose of this SOP is to outline the principles of image acquisition using HistoRx ® PM-2000, AQUA in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –
This SOP applies to routine image acquisition using AQUA within the SASoM.

3.0 Responsibilities –
It is the responsibility of all users of the AQUA within the SASoM to comply with this SOP.

4.0 Procedure –
Hardware components:
External light source - EXPO X-cite 120XL contains 120W pre-aligned lamp. Up to 1500 hours will be used. This lamp is a mercury lamp. All the users must read risk assessment and be aware of dealing with breakage of mercury lamp.

Enclosed microscope - The microscope consists of five Olympus UIS2 objective lenses (x4, x10, x20, x40, x60) and Prior precision stage with high speed PCI controller and Numerik Jena linear encoders.

Digital camera – 2048 x 2048 pixels

Dell workstation – Two dual core, hyper threaded intel processors, 2GB RAM, Windows XP Professional SP2 operating system are featured.

Joystick – used to control stage movements directly in all three axes.

Software componets:

AQUAsition	used for image acquisition
AQUAnalysis	used for image analysis

Turn Unit On:

The AQUA station consists of four main power lines plugged into one extension hub located on the rear of the monitor and EXFO lamp box. Turn on by moving switch to ON position in the extension hub.

Turn on the light source using the rocker switch at the front of the EXFO lamp unit (allow the lamp to warm up for a minimum of 20 minutes in order to stabilize the illuminator's light output.).

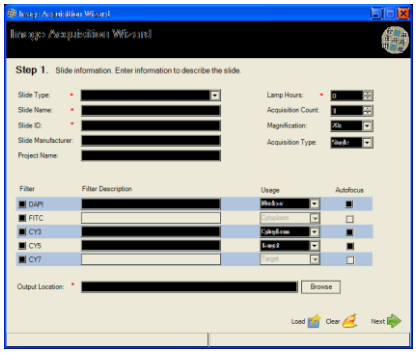
Image Acquisition(AQUAsition):

On the **Desktop**, double-click the **AQUAsition** icon to start the program.

Open the PM-2000 access doors and place a slide in the stage insert.
The specimen should face up and the label end of the slide should be towards the doors. Ensure that the slide is well secured with the retaining clip, and then close the access doors.

On the **AQUAsition Launch Bar** window, click the **Acquisition Wizard** icon to begin the slide acquisition process.

STEP1. Slide information:



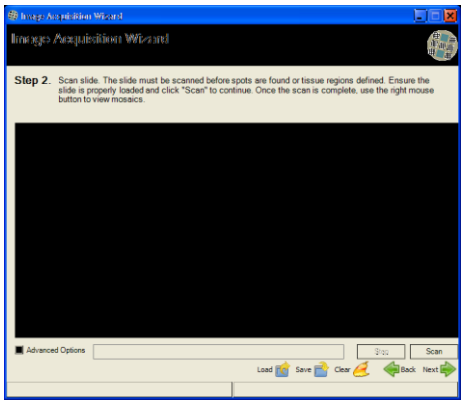
In the **Slide Type** box, select the kind of slide to be scanned.
The two available options are either a Tissue Microarray (TMA) or Whole Tissue Section (WTS). This choice will only affect the way in which the fields-of-view to be acquired are selected and will have no effect on the analysis.

On the **Slide information** window, select or fill all other relevant text boxes.
This information will be permanently attached to the image file. It can subsequently be used to assist in tracking extensive projects and can aid in the integration of the output data into a personal experiment database.

NOTE: All fields marked with a red asterisk must be filled out to continue to the next step of the slide acquisition process.

When all required fields have been completed, click the **Next** arrow to proceed to the following step.

STEP2. Scan slide:



On the **Scan slide** window, click the **Scan** button to initiate the low-resolution composite acquisition of the DAPI filter.
The low-resolution composite(s) are used in the spot finding or region selection steps only and will not be used in the quantification.

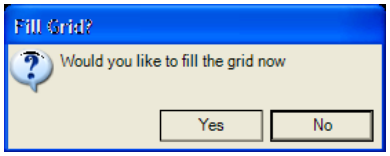
TIP: Before clicking the **Scan** button, select the **Advanced Options** checkbox to reveal additional selections allowing the opportunity to select some or all filters in use for low-resolution scanning. The composites for all filters often prove a valuable tool in a quick evaluation of the quality of the tissue sample and of the staining.

When the scan is completed, click on the **Next** arrow to proceed to the next step.

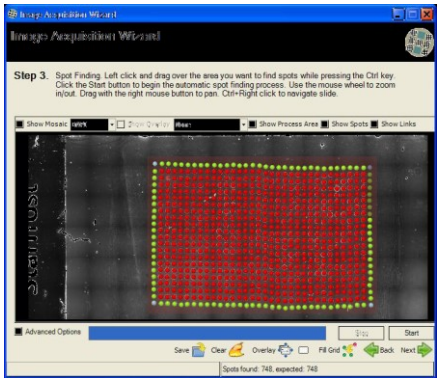
STEP3. Spot finding:

On the **Spot Finding** window, hold down **CTRL** and drag the mouse pointer to select the region of the slide containing the tissue microarray samples to be acquired.
The selected area will now be overlaid in a translucent red rectangle.

Click the **Start** button to initiate the *AQUAsition* spot finding algorithm, which will attempt to identify all tissue microarray cores within the selected region.



On the **Fill Grid** dialog box, click **Yes** to fill in missing spots based on the intersection of rows and columns.



Once a core is discovered by the algorithm, a colour-coded circle is overlaid on the composite image to mark its location. Initially, the overlaid spots will be orange, and then turn to blue, green, or red. These colours represent the spot's current position and row and column links within the array as assigned by the program. Depending on the quality of the tissue microarray and other factors, the algorithm may not be able to identify all spot array links correctly. Therefore, the colour-coding can prove very helpful for making the final adjustments to ensure proper row and column linking.

Red spot: Inner spots; directly linked to four other immediately adjacent spots (top, bottom, left, right).
Orange spot: Found spots, array links not yet determined.
Blue spot: Corner spots; directly linked to only two other spots.
Green spot: Peripheral spots; directly linked to three other spots.

On the composite image, click an area to add a missing spot or remove an incorrectly placed one.

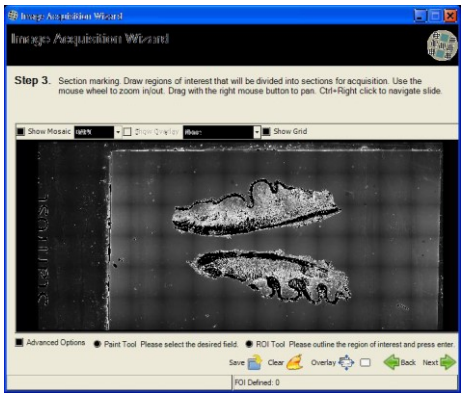
Found spots may also be repositioned by dragging. For detailed adjustments, the low-resolution composite image may be zoomed into by rotating the mouse wheel.

When all existing array cores are marked with a red, green, or blue spot, ensure that the array is rectangular by adding spots to missing row column intersections that were missed by the **Fill Grid** function.

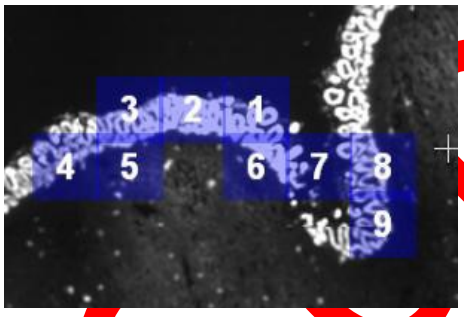
TIP: Attempting to focus on a spot location that contains no tissue can cause auto-focus failure for the offending spot as well as all those following it during high-resolution acquisition. It is recommended that spots on the array that act as placeholders for missing cores are designated as *Virtual Spots*, which will be skipped entirely during acquisition yet the spot grid integrity will be preserved. To do so, hold down **SHIFT** and click each placeholder spot.

When the spot finding is completed, click on the **Next** arrow to proceed to the next step.

STEP4. Section marking:



On the **Section marking** window, click the composite image in an area to be acquired.

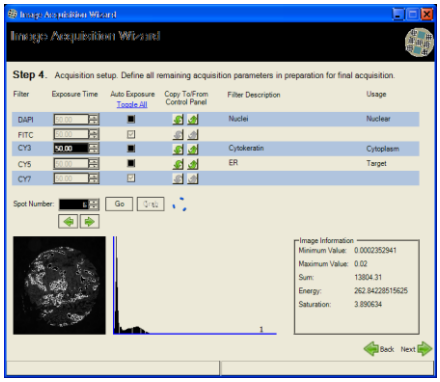


A blue, translucent field-of-view marker, its size corresponding to the magnification selected on the **Slide Information** window, will now mark the area to be acquired. To select larger areas to be acquired, drag the pointer over the areas to be acquired.

NOTE: Fields-of-view to be acquired need not be contiguous.
For increased precision, the low-resolution composite image may be zoomed into by rotating the mouse wheel and then panned around by holding down the right mouse button and dragging.

When the section marking is completed, click on the **Next** arrow to proceed to the next step.

STEP5. Image Acquisition setup:

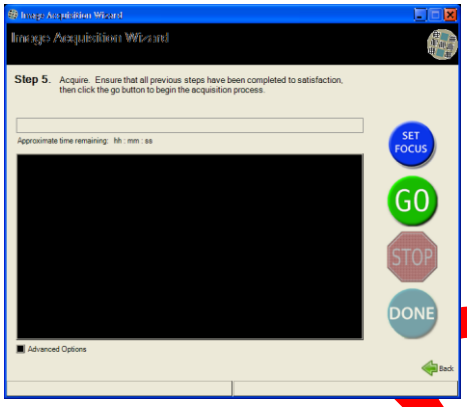


On the **Acquisition setup** window, clear the **Auto Exposure** checkboxes corresponding to the filters for which exposure times are to be manually set or else click **Toggle All** to clear the checkboxes for all filters.

*NOTE: The **Auto Exposure** function greatly enhances quantification accuracy and the dynamic range of scores by automatically adapting to the pixel intensity levels of each spot and filter. Using the manual exposure setting is not recommended for most acquisitions.*

Click on the **Next** arrow to proceed to the next step.

STEP6. Image acquire:

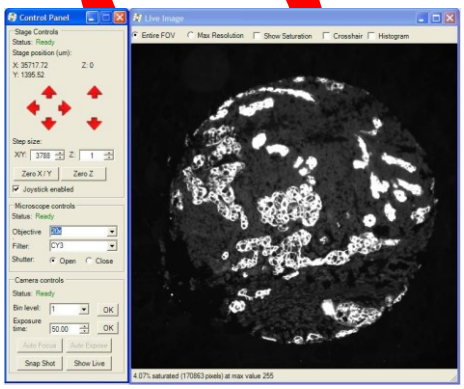


On the **Acquire** window, click **Set Focus** to set the initial focal point.
The **Live View** and **Control Panel** windows will now open and the stage will navigate to the first microarray spot or the first field of view of a whole tissue section.

NOTE: The shutter does not open automatically to avoid bleaching of the slide.

On the **Control Panel** window, click **Open** to open the shutter.

Referring to the live image displayed in the **Live View** window, bring the tissue into focus



On the **Control Panel** window, click **Open** to open the shutter.

Referring to the live image displayed in the **Live View** window, bring the tissue into focus by rotating the mouse wheel or else using the joystick.

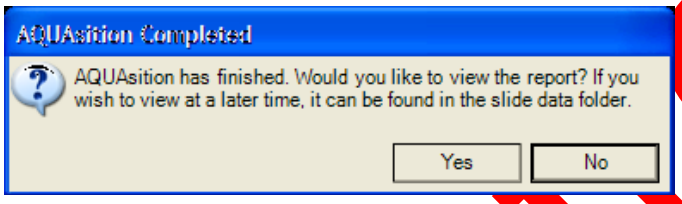
*NOTE: The mouse wheel can be used for focusing only when the **Live View** window is active.*

On the **Acquire** window, click **Go**.

The high-resolution acquisition will now proceed automatically. The **Approximate Time Remaining** counter displays an estimate of the time left to completion of acquisition. It may take up to three spots before an estimate appears and its accuracy improves with each additional spot.

Close down the viewer window in order to conserve memory on the PC. Failure to close the viewer may result in acquisition failing midway through the run. Similarly close the Settings window

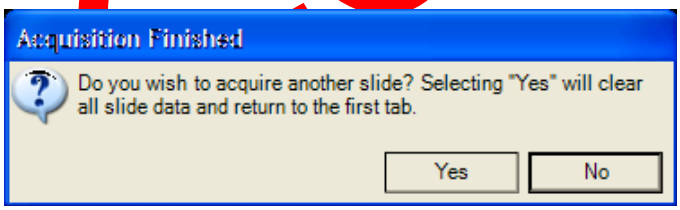
When image acquisition is completed, the shutter will close, the stage will return to its home position at the upper left corner of the slide and a notification dialog will be displayed.



Click **Yes** to view the **AQUAsition™ Results** report.
The report file is in the PDF format and will be displayed in the computer's default PDF viewer application.

Close the PDF viewer application when finished with the report.

On the **Acquire** windows, click **Done**.



On the **Acquisition Finished** dialog, click **No**.

On the **AQUAsition™ Launch Bar** window, click **Exit** on the **File** menu or click **Close** button to exit the program.

5.0 Personal protection -
Howie coat must be worn at all times.

6.0 Training
Under no circumstances should this machine be operated by anyone who is not acquainted with it or has not been trained to use it.

7.0 Related documents –

7.1 Equipment manual

7.2 Equipment Maintenance Log

7.3 Equipment Maintenance Information sheet

7.4 Risk assessments – RA/GEN/038, RA/MH/002 and COSHH/008

7.5 SOP SASoM/EQUIP/026
Image analysis using HistoRx® PM-2000, AQUA

7.6 SOP SASoM/EQUIP/024
Maintenance and replacement of EXFO lamp of the HistoRx ® PM-2000, AQUA

8.0 Approval and sign off –

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Signature: Date:

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Position: Laboratory Manager

Signature: Date:

QA release by:

Name: Alex MacLellan

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Signature:	Date: