STEM CELL LABORATORY (STCL)

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Operation of STEMvision™ Automated Colony-Forming Cell Assay Analyzer JA1

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STCL-SOP-056 JA1
OPERATION OF STEMVISION™ AUTOMATED COLONY-FORMING CELL ASSAY ANALYZER

1 PURPOSE

1.1 STEMvision™ is an automated instrument and computer system designed specifically for imaging and scoring hematopoietic colonies in the colony-forming unit (CFU) assay using optimized Methocult® media and meniscus-reading SmartDishes™.

1.2 STEMvision™ separately counts colonies derived from erythroid burst-forming units (BFU-E), myeloid progenitor cells (colony forming units granulocyte-macrophage) (CFU-GM), or multipotent progenitor cells (colony forming units granulocyte- erythrocyte-macrophage-megakaryocyte) (CFU-GEMM) that develop in conventional 14 day CFU assays.

2 INTRODUCTION

2.1 The STEMvision™ system consists of an imaging system containing a macro zoom lens, a CCD digital camera and a robotic stage that moves the culture dish over the lens. The interior temperature is controlled to prevent formation of condensation on the cultureware. A separate computer controls the imaging system using the STEMvision™ Acquisition Software which captures bright-field images of cultures and counts the colonies.

2.2 Instead of manually identifying and counting colonies using a microscope, users simply load a SmartDish™ into STEMvision™ and the instrument performs the count. Using highly sophisticated software, imaging only takes approximately 30 seconds per 35 mm well, while complete scoring analysis takes approximately 3 minutes.

2.3 STEMvision™ provides a standardized method of scoring colonies, minimizing inter-operator and inter-laboratory variability.

3 SCOPE AND RESPOSIBILITIES

3.1 The Medical Director, Stem Cell Laboratory (STCL) Manager, and designated STCL staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	CFU	Colony Forming Unit
4.2	BFU-E	Erythroid Burst Forming Unit
4.3	CFU-GM	Colony Forming Unit Granulocyte-Macrophage
4.4	CFU-GEMM	Colony Forming Unit Granulocyte-Erythrocyte-Macrophage-Megakaryocyte
4.5	mm	millimeter

4.6	STCL	Stem Cell Laboratory
4.7	N/A	Not Applicable
4.8	°C	Degrees Celsius
4.9	ID	Identification

5 MATERIALS

5.1 Reagents N/A

5.2 Supplies

5.2.1 SmartDish™ plates with plated samples

6 EQUIPMENT

6.1 STEMvision™ Instrument

6.2 STEMvision™ Acquisition software

6.3 STEMvision™ Analyzer software

6.4 Colony Marker software

7 SAFETY

7.1 Wear all appropriate personal protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to, lab coat, gloves, goggles, etc.

7.2 To reduce the risk of electrical shock, ensure that the machine is unplugged prior to accessing openings other than the enclosure door. Do not allow fluids to enter the interior of the instrument. In the event of a spill, disconnect the power cable before cleaning up. See section 7.4 of the STEMvision™ technical manual for clean-up instructions.

8 PROCEDURE

8.1 Instrument Start Up

8.1.1 Ensure the enclosure door is closed and turn STEMvision™ ON using the power switch located on the back of the instrument. A green indicator light will light up to show that the instrument is on.

8.1.2 Turn the heater ON using the switch on the front of the instrument. A red indicator light will show that the heater is turned on.

8.1.3 Allow the inner chamber to warm up to a physiological range of approximately 32–37 °C before inserting your plates. With the enclosure door closed, this will take approximately 10 minutes. This will prevent formation of condensation on the SmartDish™.

NOTE: Culture plates should not be removed from the incubator until they are ready to be put inside STEMvision™ for imaging.

8.2 Set Up of Folders for Data Storage, if needed; create a folder on the Desktop labeled "CCBB Fresh UCB"

8.2.1 Open the "CCBB Fresh UCB" folder and create a new folder labeled with the current year (i.e. 2014).

8.2.2 Open the year folder and create a new folder labeled with the year-month (i.e. 2014-10).

8.2.3 Open the year-month folder and create 2 new folders

8.2.3.1 One labeled "Data Images"

8.2.3.2 One labeled "PDF Analysis Reports"

8.3 Image Acquisition Set Up

8.3.1 Launch the Acquisition application on the computer by double clicking the icon on the desktop. Stage movement/self-calibration is indicative of a successful connection. The stage will come to a stop in the Load Position near the enclosure door.

8.3.2 A window will open displaying the Menu Bar, a Control Panel (provides quick access for controlling all major features) menu on the right and the Preview Window (shows the live camera image) on the left. See ***Figure 1***.

Figure 1

8.3.3 Click the "Load Plate" button in the Control Panel.

8.3.4 Open the enclosure door and load a SmartDish™ plate onto the sample tray. See ***Figure 2***.

NOTE: Plates should be handled with gloves to avoid creating fingerprints on the bottom of the plate that can impair image acquisition.

NOTE: Plates should be kept in the incubator until they are ready to be imaged to minimize the presence of condensation.

NOTE: If the SmartDish™ was labeled with barcodes placed on the lid, the lid will have to be removed prior to imaging.

8.3.5 Close the enclosure door.

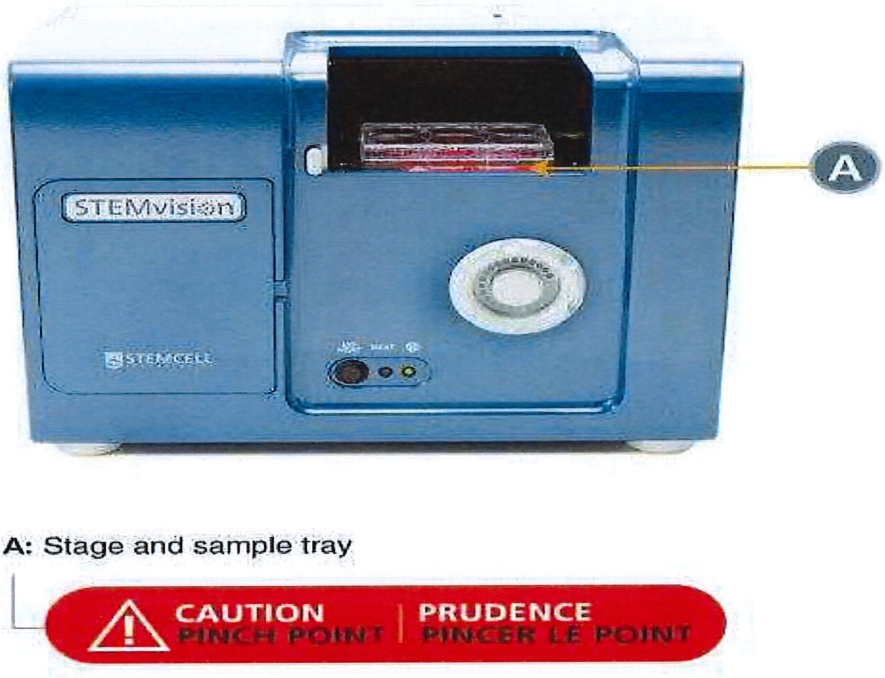

Figure 2

8.4 User, Sample and Image Identification

8.4.1 The "User ID" field in the Control Panel identifies the instrument operator for a given acquisition session.

8.4.2 Samples can be identified by Plate ID, Well ID and Sample ID.

8.4.2.1 To enter the Plate ID, click on "Plate ID" in the Menu Bar and select "Automatic". The default Plate ID name

generated is based on the acquisition date and time in the following format: YYYYMMDD-HHMinMin indicating year, month, day, hour and minutes respectively.

8.4.2.2 The Well ID is generated automatically and corresponds to the wells (A1 through B3) as seen in the Well Navigator dialog box.

8.4.2.3 For image identification, select the root folder where the acquired images, data log files and analysis files will be saved by clicking on the folder icon button in the Control Panel and selecting the root folder in the dialog box.

8.4.2.4 Each time a set of images is acquired, a new image sub-folder will be created in the root folder. The image sub-folder name corresponds to the Plate ID, whereas the image file name corresponds to the Plate ID_Well ID.jpg (ex. Plate1_A1_.jpg).

8.4.2.5 To include the Sample ID in the image file name, click on "Plate ID" in the Menu Bar and select "Include Sample ID in Image File Name". The image file name will then correspond to the Plate ID_Well ID_Sample ID.jpg (ex. Plate1_A1_Sample1.jpg).

8.5 Setting Illumination and Focus

8.5.1 Click the "Set Focus and Illumination" button in the Control Panel. When imaging multiple plates, the focus only needs to be set before the first plate is imaged.

8.5.2 Select a well by right-clicking on it in the Well Navigator dialog box.

8.5.3 Set the Illumination

8.5.3.1 Use the Joy Pad to find a location in the well that does not contain any colonies. See ***Figure 3***.

NOTE: It is important to ensure that no condensation is present on the lid of the SmartDish™ plate when setting the illumination.

8.5.3.2 Use the top slider bar to adjust the "Exposure Time" so that the peak of the exposure graph falls between the two vertical yellow lines and the "Grey Scale" value reads between 180 and 190. See ***Figure 3***.

NOTE: When the value is close to being in between 180 and 190, use the left and right arrow keys on the keyboard versus the slider bar to fine tune the value.

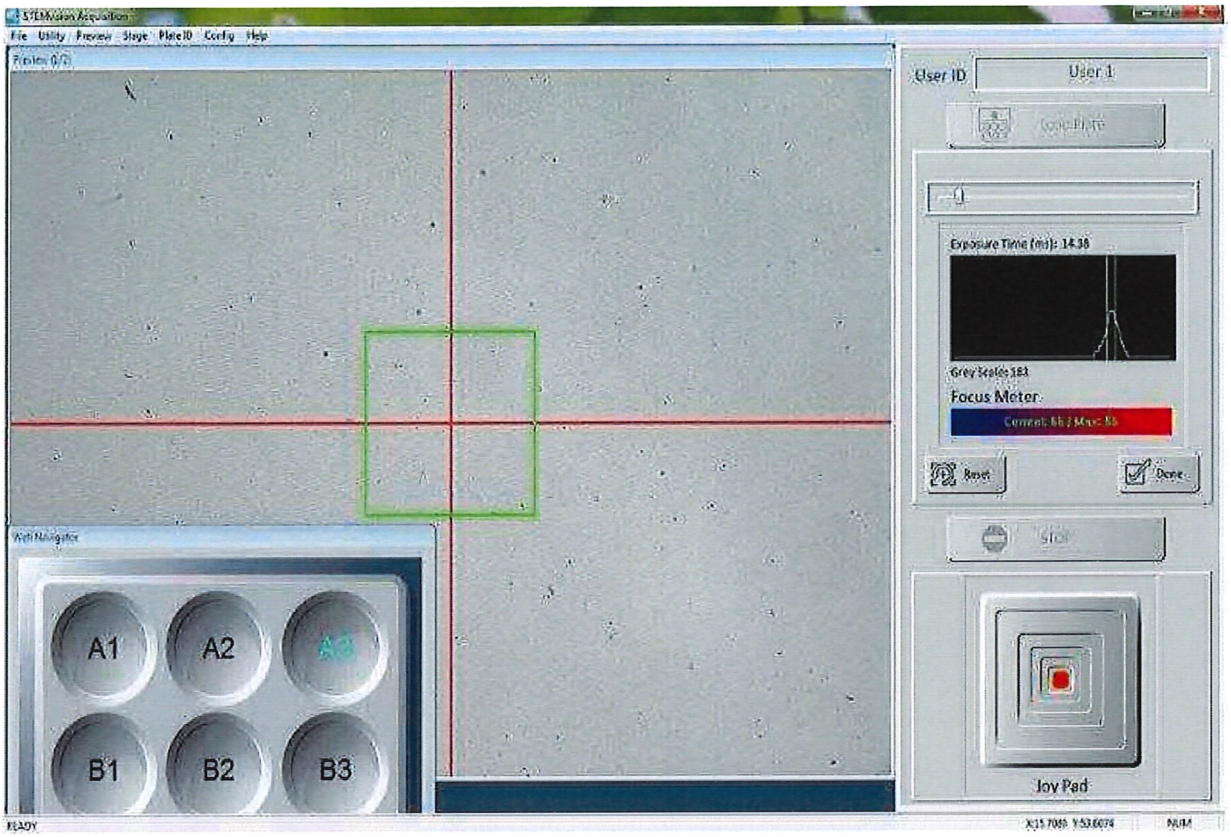

Figure 3

8.5.4 Set the Focus

8.5.4.1 Use the Joy Pad to find a location in the well where a myeloid (CFU-GM) colony with individual cells around its periphery is placed inside the focus area (the green box in the Preview Window). See ***Figure 4***.

8.5.4.2 Turn the coarse or fine focus knob. The live preview image will move in and out of focus. This is necessary in order for the software to identify the optimal focal plane. Once a range of focal planes have been "seen", the live graph will show optimal focus as a Current/Max value of 1.0 and poor focus as a value far away from 1.0.

8.5.4.3 Use the focus knobs – coarse (outer ring, turning clockwise, then fine focus (inner knob), turning counter-clockwise to adjust the focus so that the red line in the live graph approaches a value of 1.0.

8.5.4.4 Click the "Done" button. See ***Figure 4***.

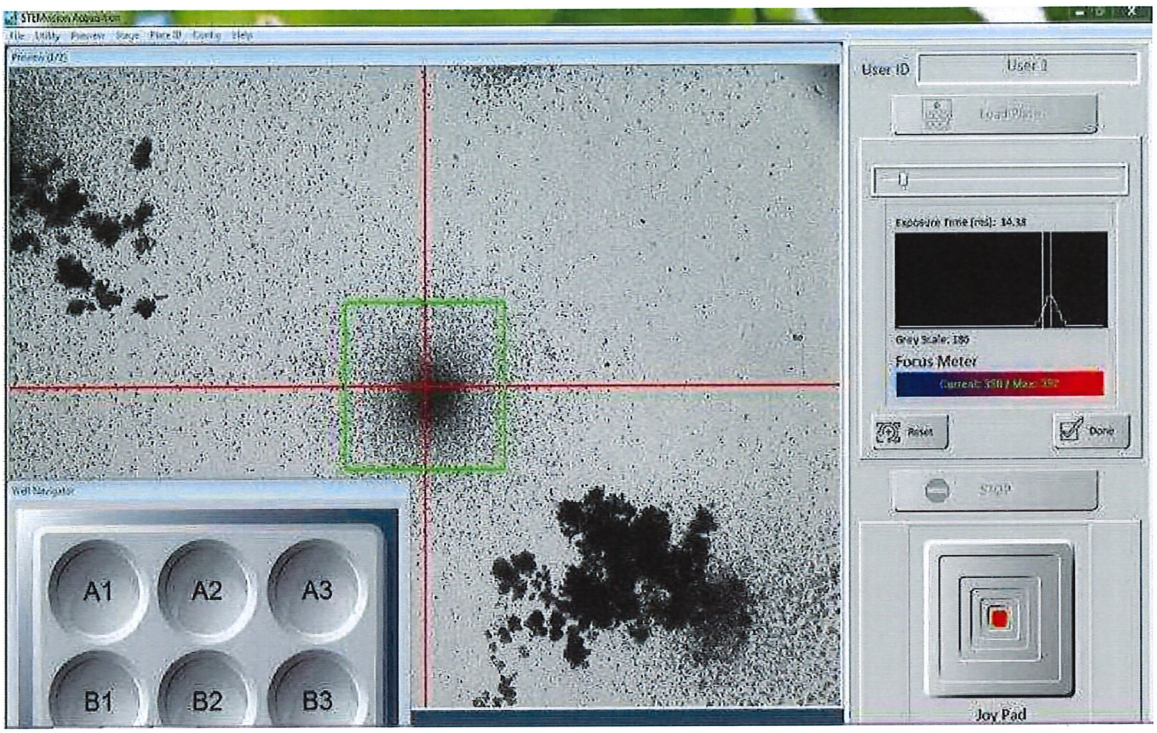

Figure 4

8.6 Image Acquisition

8.6.1 To obtain a smaller printable version of the analyzed image, click on "Options" in the Menu Bar and select "Result Image". This file will be saved in the same sub-folder as the images. Once selected, "Result Image" will be set to default for future analysis.

8.6.2 **IMPORTANT:** If printed report forms will be required, sample information must be entered before image acquisition. Sample ID information will need to be entered separately prior to the imaging of each new plate.

8.6.2.1 The Sample ID is entered manually in the "Sample ID Information" dialog box.

8.6.2.2 Click on "Plate ID" in the Menu Bar and select "Sample ID Information".

8.6.2.3 Populate the fields of the "Sample ID Information" dialog box with the relevant information. See ***Figure 5***.

8.6.2.4 Click the "Add/Modify this Sample ID" button when finished populating the fields. The sample will appear on the left side of the dialog box under "Sample ID List". See ***Figure 5***.

8.6.2.5 Continue adding the next Sample ID information for that plate as required.

8.6.2.6 Click the "Done" button when both samples to be imaged have been added to the list. See ***Figure 5***.

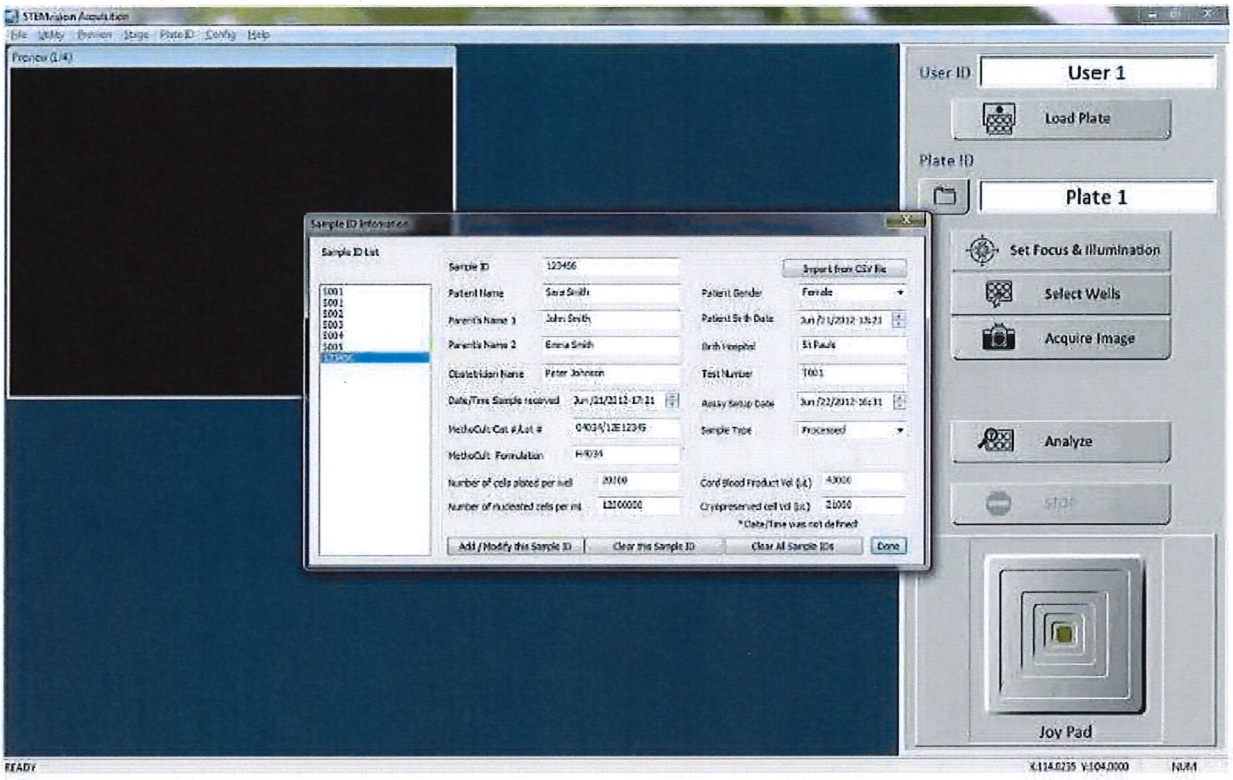

Figure 5

8.6.3 Selecting Wells to be Imaged

8.6.3.1 Click the "Select Wells" button in the Control Panel. A dialog box will appear on the screen showing a graphic of a 6-well plate and a "Well Info" table where the associated sample information is displayed. See ***Figure 6***.

8.6.3.2 Select all applicable replicate wells by clicking on the 6-well plate graphic. As wells are selected, the matching lines on the "Well Info" table are highlighted. See ***Figure 6***.

8.6.3.3 Select a previously defined Sample ID for the selected well(s) by using the drop-down menu. See ***Figure 6***.

8.6.3.4 Click the "Apply" button when all the desired information has been entered. The highlighted wells on the "Well Info" table and 6-well plate graphic will be assigned a new color. See Figure 6.

8.6.3.5 Repeat steps 8.5.2.2-8.5.2.4 until the data for all wells to be imaged has been entered in the "Well Info" table.

8.6.3.6 Click the "Done" button when finished. See ***Figure 6***.

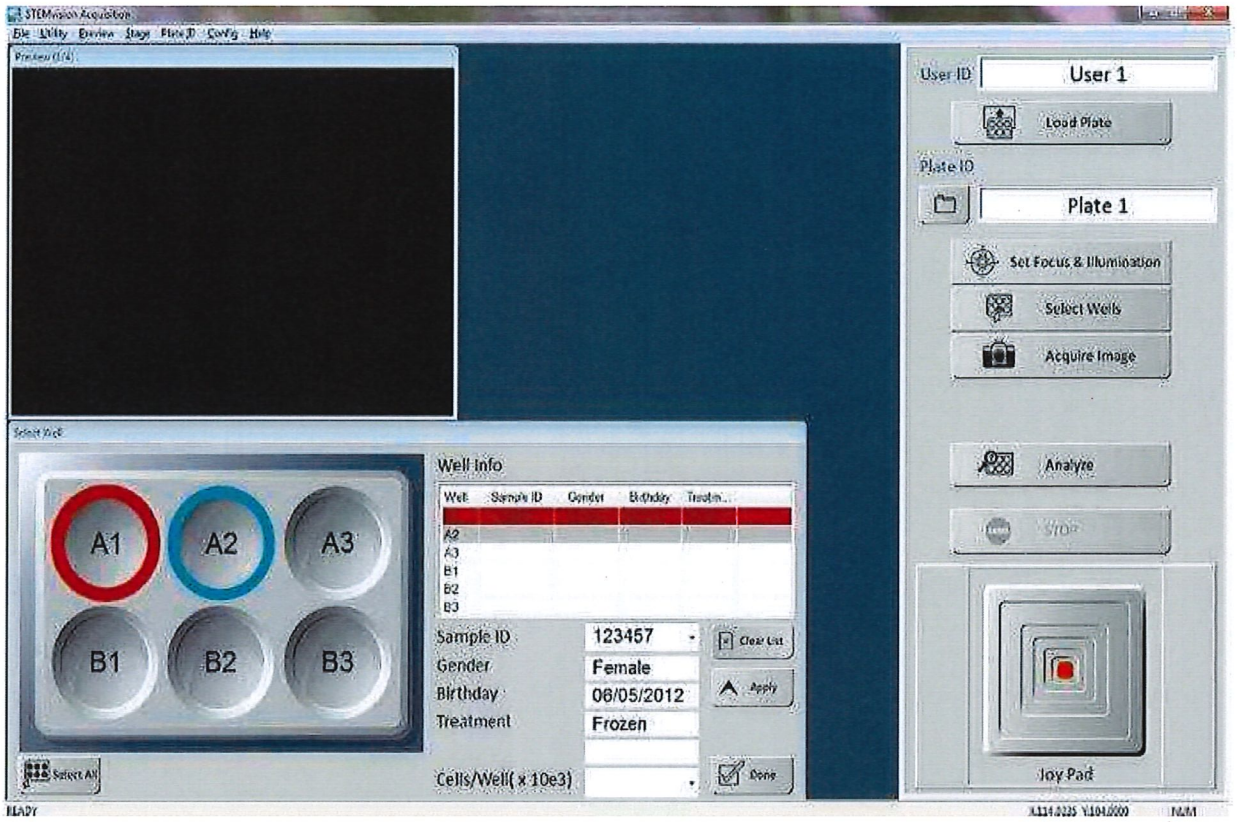

Figure 6

8.6.4 Acquiring Images

8.6.4.1 Click the "Acquire Image" button in the Control Panel.

8.6.4.2 Once image acquisition has been completed for all desired wells, open the STEMvision™ enclosure door and remove the SmartDish™ plate from the sample tray.

8.6.4.3 **NOTE:** Each well is imaged in approximately 30 seconds and an entire SmartDish™ 6-well plate is imaged in approximately 3 minutes.

8.6.4.4 To image additional plates, load the next SmartDish™ and acquire images as described in steps 8.5.3.1-8.5.3.2.

8.6.4.5 After images of all cultures have been acquired, shut down the STEMvision™ instrument.

8.7 STEMvision™ Shut Down

8.7.1 Turn OFF the heater using the switch located on the front of the instrument.

8.7.2 Turn OFF STEMvision™ using the power switch located on the back of the instrument.

8.8 Image Analysis

8.8.1 Start the STEMvision™ Analyzer application on the computer by double clicking the icon on the desktop.

Note: Launching the Analyzer application can also be performed prior to shut down of the STEMvision™ by clicking on the "Analyze" button on the control panel of the Acquisition software.

8.8.2 Selecting Images for Analysis

8.8.2.1 Click the "Select Files" button and then browse and select the sub-folder that contains the images to be analyzed. All acquired image files in the selected folder will appear under the "Input Image" field. See ***Figure 7***.

8.8.3 Select CB for "Assay Type", Day-14 for day of scoring and without EPO for assay condition from the drop-down menu. See Figure 7.

8.8.4 Click the "Process" button to start data analysis. A progress window will show the images being analyzed and their progress. See ***Figure 7***.

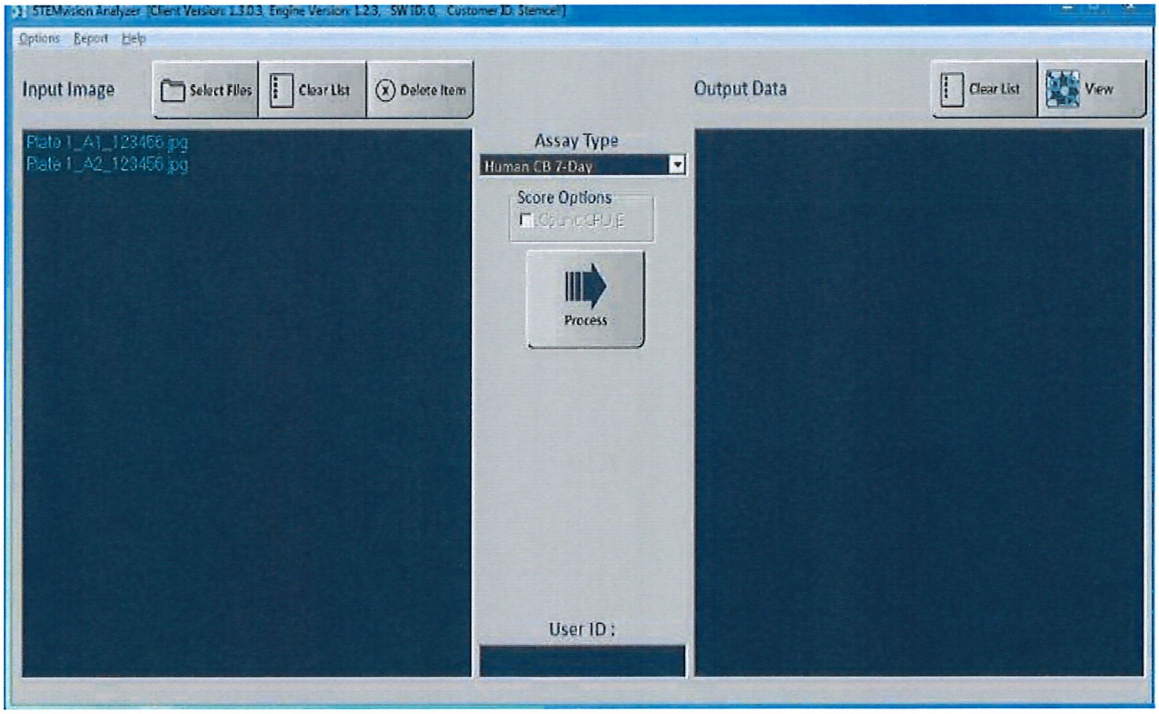

Figure 7

8.8.5 As the images are analyzed the file names will move from the "Input Image" to the "Output Data" field. The analysis result files will be saved in the same sub-folder as the images. See ***Figure 8***.

8.8.6 Click the "Clear List" button to clear the list of analyzed images. This removes files from the "Output Data" list only; all data files will remain stored in their original location. See ***Figure 8***.

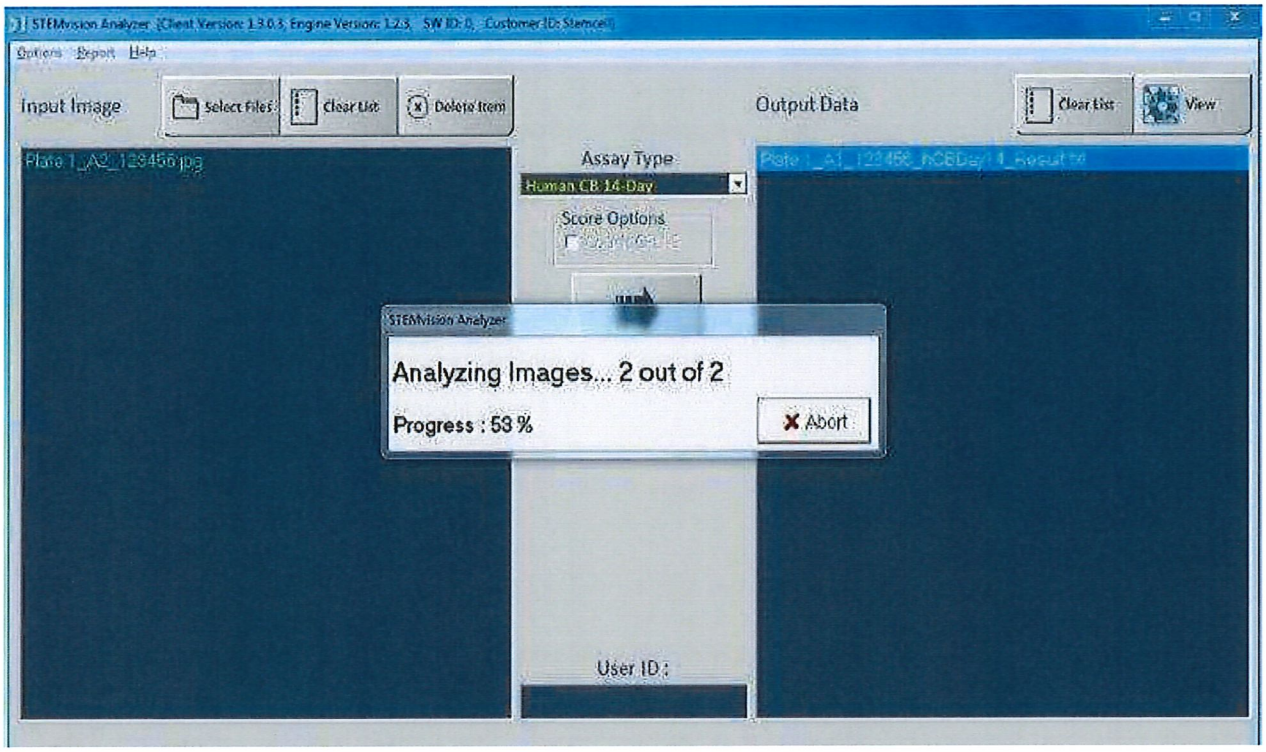

Figure 8

8.9 Viewing and Printing Results

8.9.1 Reports will automatically print to the dedicated printer post image analysis. Ensure the "Auto Print" is selected in the Menu Bar at the time of analysis.

8.9.2 To Generate a CFU assay report at a later date:

8.9.2.1 In the Analyzer application, select the appropriate "Assay Type" corresponding to the tissue, day of scoring (Day-14) and assay condition form the drop-down menu.

8.9.2.2 Click on "Report" in the Menu Bar and select "Find"

8.9.2.3 Enter the Sample ID in the search field of the dialog box and click the "Find" button.

8.9.2.4 Click the "Print" button to directly print the report form.

8.9.2.5 Click "Done" once finished.

9 RELATED DOCUMENTS AND FORMS

9.1 STCL-SOP-056 STEMvision™ Automated Colony Counting and Enumeration of Hematopoietic Progenitors in Fresh Umbilical Cord Blood

9.2 STCL-SOP-056 JA2 STEMvision™ Automated Colony-Forming Unit (CFU) Assay Reader Technical Manual

10 REFERENCES

10.1 N/A

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
02	Barbara Waters-Pick	Updated steps associated with Section 8.5.4 "Set the Focus"

Signature Manifest

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