# STEM CELL LABORATORY (STCL)

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TECHNICAL MANUAL

# STEMvision™

## Automated Colony-Forming Unit (CFU) Assay Reader

STEMCELL™ TECHNOLOGIES

## Table of Contents

# 1.0 Safety Information and Warnings

STEMvision™ Instrument (Catalog # 22000) must be operated by trained professionals and is intended for research use only. Not intended for human or animal diagnostic or therapeutic uses.

For operation, STEMvision™ should be placed on a clean, flat surface free of any foreign objects or debris and insulated from excessive vibration. There should be at least 10 cm (4 in) of clearance on each side of the instrument.

Always connect the power supply to a 3-prong, grounded AC outlet rated 120V/240V, 60Hz/50Hz, 1.5A, 1Ph using the AC power cord provided with STEMvision™. Before plugging the instrument in, be sure that the fuse rated F 2A L 250VAC is installed.

Under normal operating conditions, it is not necessary to open any part of the STEMvision™ other than the enclosure door. To reduce the risk of electrical shock, ensure that the instrument is unplugged prior to accessing openings other than the enclosure door.

Do not allow fluids to enter the interior of the STEMvision™. In the event of such a spill, disconnect the power cable before cleaning. See Section 7.4 for spill clean-up instructions.

Operate STEMvision™ at 15 - 30°C, in an environment with a relative humidity of 20 - 85%. STEMvision™ should be used indoors only. STEMvision™ does not require placement in a biohazard safety cabinet and is not specified for use inside an incubator or cold room.

STEMvision™ weighs 27 kg (59 lb). Exercise caution when moving STEMvision™. It is recommended that two people are present to lift or move the instrument safely.

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

In case of malfunction, call STEMCELL Technologies Inc. for service. Servicing performed by anyone other than a STEMCELL Technologies representative will void the warranty associated with STEMvision™. There are no user-serviceable parts inside the instrument and the outer casing of STEMvision™ should never be removed.

STEMvision™ is manufactured and distributed by STEMCELL Technologies Inc., 570 West 7th Avenue, Suite 400, Vancouver BC, Canada V5Z 1B3. Call 1-604-877-0713 or toll-free 1-800-667-0322 within North America.

# 2.0 Introduction

STEMvision™ is an automated instrument and computer system that is designed specifically for imaging, classifying (or "scoring") and counting hematopoietic colonies produced by human progenitor cells in the colony-forming unit (CFU) assay. It separately counts colonies derived from erythroid progenitor cells (colony-forming units-erythroid [CFU-E] and burst-forming units-erythroid [BFU-E]), uni- or bi-potent myeloid progenitor cells (colony-forming units granulocyte-macrophage [CFU-GM], CFU-G and CFU-M), or multi-potent progenitor cells (colony-forming units granulocyte, erythrocyte, macrophage, megakaryocyte [CFU-GEMM]) that develop in conventional 14-day CFU assays of bone marrow (BM), mobilized peripheral blood (MPB) or umbilical cord blood (CB) cells. STEMvision™ can also count the total number of colonies in a faster 7-day CFU assay of CB cells that has been developed for CB banks. A summary of STEMvision™ Analysis Packages used for different human CFU assays is described in Table 1.

STEMvision™ is designed for use with MethoCult™ media (see Table 1 for the appropriate MethoCult™ formulation for different STEMvision™ CFU assays) and meniscus-reducing SmartDish™ cultureware (Catalog #27301/27302). STEMvision™ is optimized for analysis of CFU assays that do not contain background red blood cells (RBCs). Red cells must be cleared from fresh blood samples using HetaSep™ (Catalog #07906) before setting up the CFU assay. For complete instructions on how to set up CFU assays, refer to the Technical Manual: Human Colony-Forming Unit (CFU) Assays Using MethoCult™ (Document #28404) available at www.stemcell.com or contact us to request a copy.

STEMvision™ eliminates the inter- and intra-individual and laboratory variation associated with manual colony scoring by using sophisticated image acquisition and analysis software to identify and classify hematopoietic colonies. The morphological criteria for classifying the different sub-types of CFUs are applied consistently, facilitating standardization of the CFU assay and ensuring accurate and reproducible results. Each 35-mm culture is imaged in approximately 30 seconds and an entire SmartDish™ 6-well plate is imaged in approximately 3 minutes. Analysis can be completed offline in about 20 minutes per plate. For high throughput processing, 20 plates containing 120 cultures can be imaged in about 80 minutes, with the image analysis executed overnight. Digital images of the entire culture can be permanently archived for future reference.

The results of 7-day and 14-day CFU assays produced by STEMvision™ can be printed out in two different report formats. A "Lab" format report contains detailed information on the CFU assay for the laboratory or transplant physician. A second "Parent" report form is suitable for presentation of the CFU assay results to parents banking their child's CB with a private bank for potential future autologous use.

In the following sections, each of these different aspects of using STEMvision™ will be described in detail.

Prior to using STEMvision™, please ensure that you have read Section 1.0: Safety Information and Warnings.

**Table 1: Summary of STEMvision™ Assays and MethoCult™ Media for Identifying Different Human CFU Types**

| TISSUE | CFU ASSAY DURATION | COLONY SUB-TYPES IDENTIFIED BY STEMvision™ | MethoCult™ MEDIUM | STEMvision™ ANALYSIS PACKAGE | STEMvision™ ASSAY TYPE FIELD** | PRINTED CFU ASSAY REPORT FORM |
|---|---|---|---|---|---|---|
| Cord Blood (CB) | 7 Days | Total CFUs without distinction of colony types | MethoCult™ Express (Catalog #04437/04447) | STEMvision™ Human Cord Blood 7-Day CFU Analysis Package (Catalog #22001) | Human CB 7-Day | Lab Parent |
| Cord Blood (CB) | 14 Days | BFU-E CFU-G/M/GM CFU-GEMM | MethoCult™ H4034 Optimum (Catalog #04034/04044 | STEMvision™ Human Cord Blood 14-Day CFU Analysis Package (Catalog #22002) | Human CB 14-Day | Lab Parent |
| | | CFU-G/M/GM | MethoCult™ H4035 Optimum without EPO (Catalog #04035/04045) | | Human CB 14-Day EPO-Free | |
| Bone Marrow (BM) | 14 Days | BFU-E CFU-E* CFU-G/M/GM CFU-GEMM | MethoCult™ H4034 Optimum (Catalog #04034/04044) | STEMvision™ Human Bone Marrow 14-Day CFU Analysis Package (Catalog #22003) | Human BM 14-Day | Lab |
| | | CFU-G/M/GM | MethoCult™ H4035 Optimum without EPO (Catalog #04035/04045) | | Human BM 14-Day EPO-Free | |
| Mobilized Peripheral Blood (MPB) | 14 Days | BFU-E CFU-G/M/GM CFU-GEMM | MethoCult™ H4034 Optimum (Catalog #04034/04044) | STEMvision™ Human Mobilized Peripheral Blood 14-Day CFU Analysis Package (Catalog #22004) | Human MPB 14-Day | Lab |
| | | CFU-G/M/GM | MethoCult™ H4035 Optimum without EPO (Catalog #04035/04045) | | Human MPB 14-Day EPO-Free | |

Abbreviations: BFU-E: Burst-Forming Unit-Erythroid; CFU-E: Colony-Forming Unit-Erythroid; CFU-G/M/GM: Colony-Forming Unit-Granulocyte/Macrophage/Granulocyte-Macrophage; CFU-GEMM: Colony-forming Unit-Granulocyte, Erythrocyte, Macrophage, Megakaryocyte; EPO: erythropoietin; BM: bone marrow; MPB: mobilized peripheral blood; CB: cord blood.

*Counting of CFU-E is optional.

**Assay types are selected by the user when using the STEMvision™ Analysis application

# 3.0 STEMvision™ System

The STEMvision™ instrument consists of an imaging module that enables the acquisition of high resolution composite images of hematopoietic colony assays plated in SmartDish™ cultureware (each plate comprising six 35 mm wells). Key components of the instrument include a macro zoom lens, a CCD digital camera and a robotic stage that moves the culture plate over the lens in increments as images are acquired. In addition, the interior temperature of the acquisition chamber is warmed to prevent the formation of condensation on the inside of the SmartDish™ lid.

The imaging system is controlled from a separate computer, provided with the instrument.

*Note: The computer provided with STEMvision™ does not contain anti-virus software. Users are encouraged to check with their own IT department to determine what anti-virus software is required or recommended at their institution.*

The software applications included in the STEMvision™ system are:

- **STEMvision™ Acquisition** - for acquiring bright-field images of CFU assay cultures
- **STEMvision™ Analyzer** - for analyzing culture images using CFU assay algorithms specific for different hematopoietic colony and tissue types
- **STEMvision™ Colony Marker** - for viewing, and if desired, manual scoring and editing of automated results

STEMvision™ has been optimized for use with SmartDish™ cultureware and MethoCult™ media. Use with different cultureware or media that has not been tested and approved by STEMCELL Technologies is not recommended. Refer to Table 1 for a summary of the different types of CFU assays and recommended MethoCult™ media that are compatible with STEMvision™. For use with other MethoCult™ formulations, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

## 3.1 Unpacking and Set up

A STEMCELL Technologies Inc. representative or trained individual will unpack and set up STEMvision™ in your laboratory. They will connect the STEMvision™ instrument to the computer and verify the stage calibration, function and positioning.

*Note: Stage positioning can be verified using the calibration plate provided with STEMvision™ (see Section 7.2). Adjustments should only be performed by STEMCELL Technologies' qualified personnel.*

## 3.2 Start Up

1. Ensure that the STEMvision™ enclosure door (Figure 1F) is closed.
2. Turn ON STEMvision™ using the power switch located on the back of the instrument (Figure 2A).

   *Note: A green indicator light on the front of the instrument (Figure 1C) will show that the instrument is turned on.*
3. Turn ON the heater using the switch located on the front of the instrument (Figure 1B). Proper pre-warming of the internal chamber will prevent the formation of condensation on the SmartDish™ plate lid.

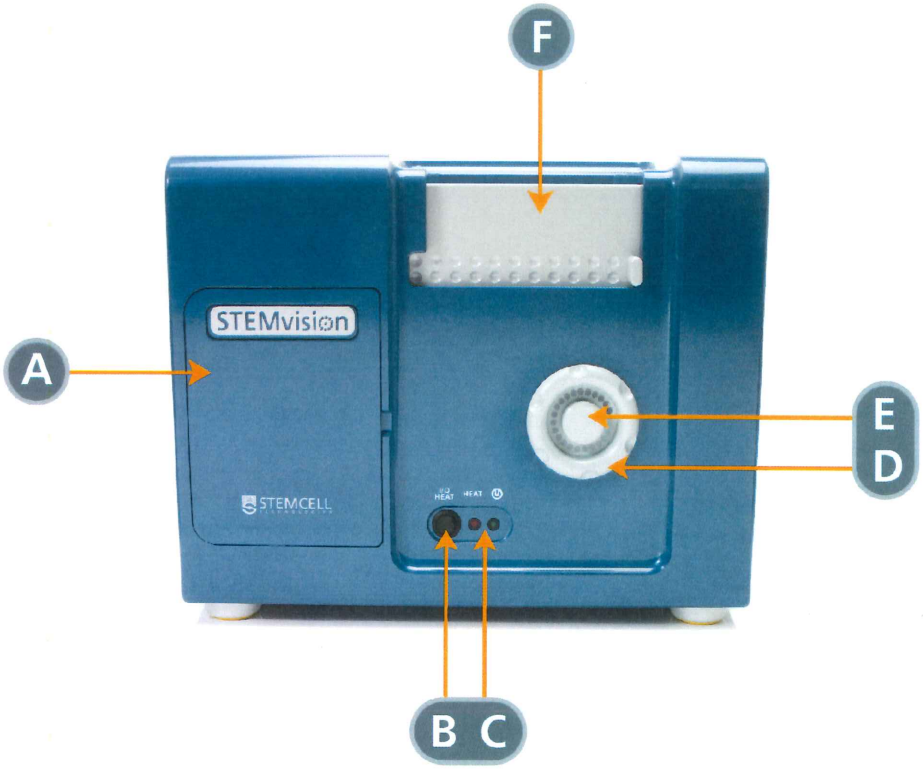
   *Note: A red indicator light on the front of the instrument (Figure 1C) will show that the heater is turned on.*
4. Allow the inner chamber to warm up for about 10 minutes before inserting a SmartDish™ plate. Cultures should not be removed from the incubator until they are ready to be put inside STEMvision™ for imaging.

   *Note: STEMvision™ is not specified for use as an incubator.*
5. Proceed with image acquisition (see Section 4.0)

## 3.3 Shut Down

1. Turn OFF the heater using the switch located on the front of the instrument (Figure 1B).
2. Turn OFF STEMvision™ using the power switch located on the back of the instrument (Figure 2A).

   *Note: Both indicator lights on the front of the instrument (Figure 1C) will turn off.*




**A:** Lens access door

**B:** Heater on/off switch

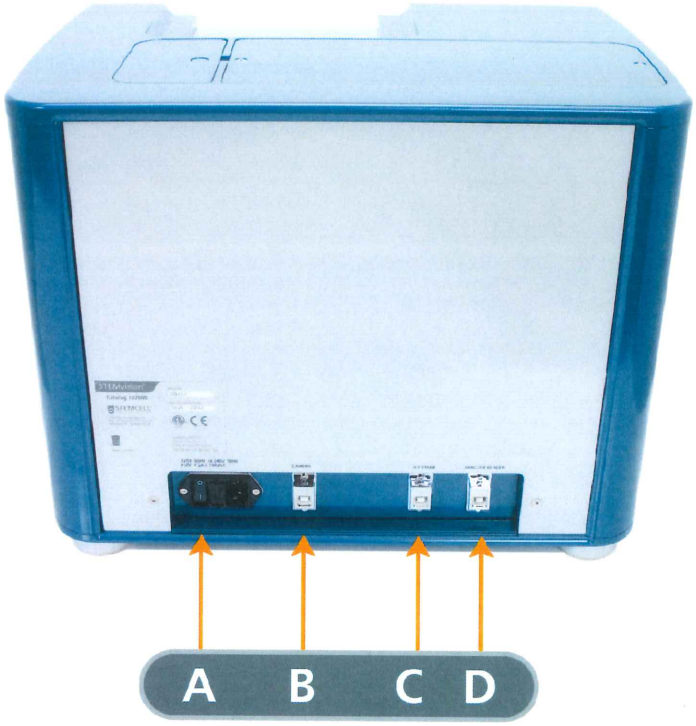
**C:** Indicator lights
– Red for Heater Power; Green for Main Power

**D:** Coarse focus knob

**E:** Fine focus knob

**F:** Enclosure door

**Figure 1: Front View of the STEMvision™ Instrument.**



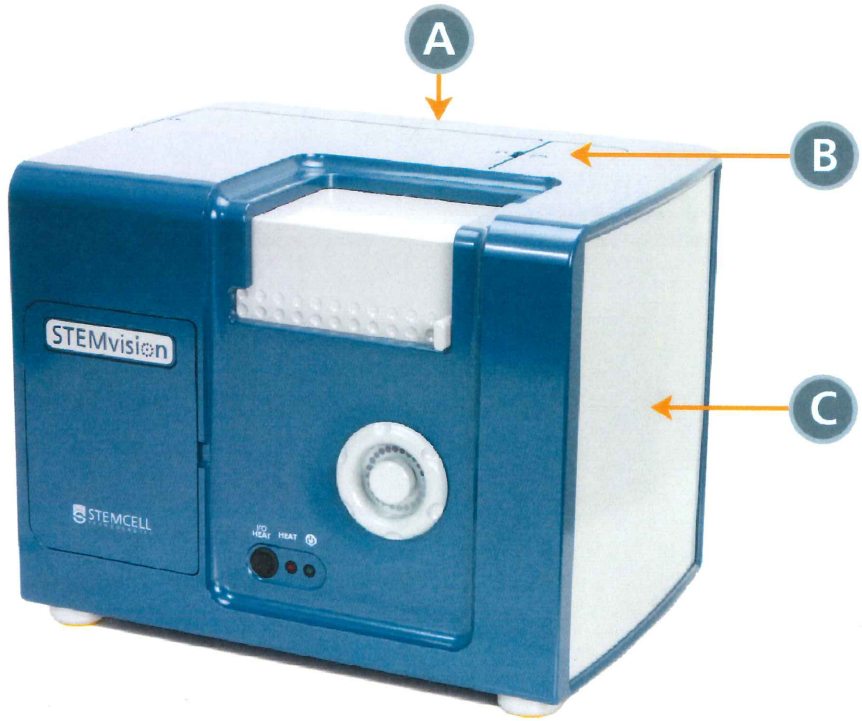

**A:** Power entry module, fuse rating and power switch

**B:** USB camera connector

**C:** USB XY stage connector

**D:** USB barcode reader connector

**Figure 2: Back View of the STEMvision™ Instrument.**




**A:** Optics door

**B:** Potentiometer door (label located under the cover)


**C:** Side panel

**Figure 3: Top and Side View of the STEMvision™ Instrument.**

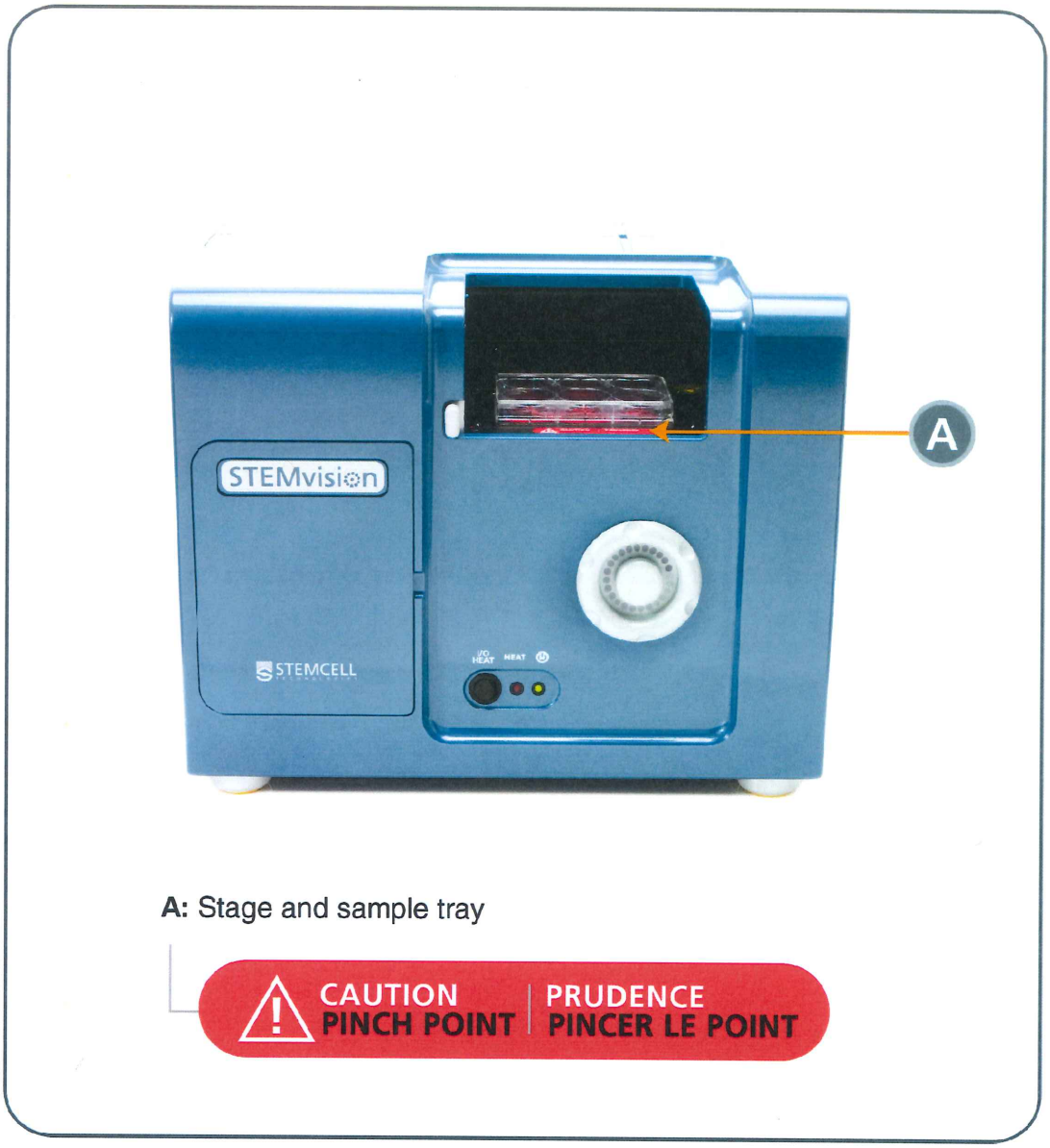



**Figure 4: Front View of the STEMvision™ Instrument with the Enclosure Door Open.**

# 4.0 STEMvision™ Acquisition Application

The STEMvision™ Acquisition application is used to set up and control all aspects of CFU assay image acquisition. The application can only be run when the STEMvision™ instrument is turned on.

*Note: Do not turn off the STEMvision™ instrument while the STEMvision™ Acquisition application is running. If the instrument is accidentally turned off, re-initialize the instrument by clicking on "Stage" in the Menu Bar and selecting "Initialize". Alternatively, restart the application, however, any Sample ID information will need to be re-entered.*

## 4.1 User Interface Overview

1. Turn ON the STEMvision™ instrument as described in Section 3.2.
2. Start the STEMvision™ Acquisition application on the computer (double-click the icon on the desktop). The user interface of the STEMvision™ Acquisition application is shown in Figure 5.

   *Note: At initial start-up of the application, the stage will complete a self-calibration routine. This movement of the stage is normal.*

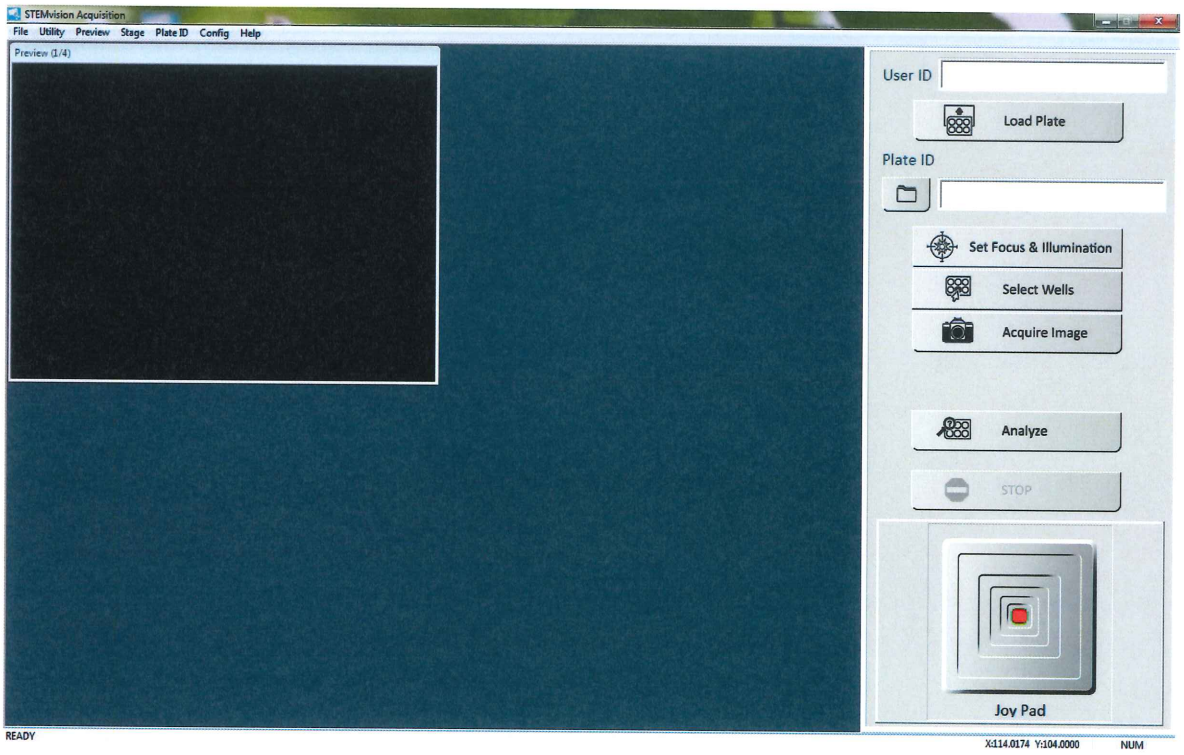



**Figure 5: The STEMvision™ Acquisition Application upon Start Up.** The main Control Panel menu is on the right and the Preview Window is on the left.

- **Menu Bar:** Located along the top of the application window. It provides access to additional instrument settings.
- **Preview Window:** Located on the upper left side of the application window. The live camera image is shown in this window. The preview window can be viewed at two different resolutions, click on "Preview" in the Menu Bar and select "Full" to view a larger preview at higher resolution.
- **Control Panel:** The entire grey area located on the right side of the application window. It provides quick access for controlling all major features of the application, and fields for unique sample and user/operator identification.

## 4.2 Acquisition Set up

1. Click the "Load Plate" button in the Control Panel.
2. Open the STEMvision™ enclosure door (Figure 1F) and load a SmartDish™ plate onto the sample tray (Figure 4A). It is very important to ensure that the plate is properly inserted and sits completely flat on the tray.

   *Note: SmartDish™ plates should be handled with gloves to avoid creating fingerprints on the bottom of the plate that can impair image acquisition.*
3. Close the STEMvision™ enclosure door.

   *Note: If the STEMvision™ enclosure door is opened during acquisition, the STEMvision™ Acquisition application will stop the acquisition process. To restart image acquisition, close the enclosure door as instructed by the on-screen warning, and then repeat the image acquisition procedure as described in Section 4.6.*

## 4.3 Controlling Stage Position

The stage located inside the instrument moves the sample tray on which the SmartDish™ is placed. There are different ways to move the position of the tray and SmartDish™:

- **Moving to the Center of the Stage:** Click on "Stage" in the Menu Bar and select "Center". This will center the stage and will place the SmartDish™ in close proximity to the heater. This will minimize the formation of condensation on the SmartDish™ lid.
- **Moving to a Specific Well:** Click on "Utility" in the Menu Bar and select "Well Navigator". A new dialog box (Well Navigator) will appear showing a schematic image of the SmartDish™ plate. Right-click on the desired well and the stage will move to the center of the selected well.

  *Note: The selected well will be highlighted in turquoise in the Well Navigator dialog box window and its live image will be shown in the Preview Window.*
- **Using the Joy Pad:** The Joy Pad is located at the bottom of the Control Panel. To use it, click and drag the red dot of the Joy Pad in the desired direction. The live image will be displayed in the Preview Window.

  *Note: Moving the red dot away from the center of the Joy Pad increases the speed of the stage movement.*
- **Entering XY Coordinates:** The position of the stage is displayed as XY coordinates at the bottom of the Control Panel. These coordinates indicate the position of the stage in millimeters relative to the origin of the stage's calibrated range of travel. Click on "Stage" in the Menu Bar and select "Move To", then enter the desired XY coordinates in the new dialog box. Click on "Move" to automatically move the stage to the specified XY coordinates.

## 4.4 User, Sample and Image Identification

### 4.4.1 User Identification

The "User ID" field in the Control Panel identifies the instrument operator for a given acquisition session. Entering a User ID is optional. If entered, the User ID is recorded in the acquisition log file (see Section 6.6).

### 4.4.2 Sample Identification

Each sample can be identified by: Plate ID, Well ID and Sample ID. This information is then used in naming acquired images (Section 4.4.3), generating log files (Section 6.6), and/or "Lab" and "Parent" CFU assay report forms (Section 6.1).

*Note: When manually entering Plate ID and Sample ID information, avoid characters not compatible with the Microsoft® Windows® file naming system, such as "/", "\", "*", and "?".*

**Plate ID:**

Automatic Entry - Click on "Plate ID" in the Menu Bar (at the top of the application window) 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 (e.g. 20121103-1231 for November 3, 2012 at 12:31 PM).

Manual Entry - Enter any name in the "Plate ID" field in the Control Panel.

Barcode Entry - Ensure the SmartDish™ is loaded in the instrument so that the barcode is facing the right-hand side of the instrument.

1. Click on "Config" in the Menu Bar and select "Internal Barcode Reader" (indicated by a check mark).
2. Click on "Plate ID" in the Menu Bar and select "Barcode".
3. Click the "Load Plate" button in the Control Panel. STEMvision™ will automatically scan the SmartDish™ barcode.

The Plate ID name generated by this procedure corresponds to the SmartDish™ serial number from the barcode number.

**Well ID:**

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

**Sample ID:**

The Sample ID information may be imported into the STEMvision™ Acquisition application via a CSV file, or entered manually in the "Sample ID information" dialog box, when generating printed "Lab" or "Parent" CFU assay report forms.

Automatic Entry from a Pre-Populated CSV File

Sample information must be entered and saved in a CSV file before starting image acquisition. An example of a CSV file with 18 comma separated columns is located on the desktop of the computer.

1. Ensure a CSV file is populated with data and formatted correctly. The file must consist of 18 comma separated columns. Refer to Table 2 for guidelines on how to enter information in the file.

**Table 2: Column Properties in the CSV file.**

| COLUMN | INFORMATION TRACKED IN COLUMNS | FORMAT | EXAMPLE |
|---|---|---|---|
| A | Sample ID | 30 characters max | 123456 |
| B | Patient Name | 30 characters max | Sara Smith |
| C | Parent Name 1 | 30 characters max | John Smith |
| D | Parent Name 2 | 30 characters max | Emma Smith |
| E | Gender | -1 (Not Defined)<br>0 (Female)<br>1 (Male) | 0 |
| F | Birth Date/Time | month/day/year hh:min in numeric format (hours in 24h format) | 6/21/2012 13:21 |
| G | Birth Hospital | 30 characters max | St Pauls |
| H | Obstetrician Name | 30 characters max | Peter Johnson |
| I | Test Number | 30 characters max | T001 |
| J | Number of Nucleated Cells per mL | Numeral | 12000000 |
| K | Number of Cells Plate per Well | Numeral | 20000 |
| L | Cryopreserved Cell Volume in µL | Numeral | 21000 |
| M | Blood Product Volume in µL | Numeral | 43000 |
| N | Sample Type | -1 = Not Defined<br>0 = Fresh<br>1 = Frozen<br>2 = Whole<br>3 = Processed | 3 |
| O | Assay Setup Date/time | month/day/year hh:min in numeric format (hours in 24h format) | 6/22/2012 16:31:00 PM |
| P | Sample Received Date/time | month/day/year hh:min in numeric format (hours in 24h format) | 6/21/2012 17:21:00 PM |
| Q | MethoCult™ Formulation | 30 characters max | H4034 |
| R | MethoCult™ Catalog Number | 30 characters max | 04034/12E12345 |

2. Click on "Plate ID" in the Menu Bar and select "Sample ID Information".
3. Click the "Import from CSV file" button in the new dialog box and browse to select the populated CSV file. The list of samples extracted from the CSV file will appear on the left side of the dialog box window under "Sample ID List" (Figure 6).

   *Optional: Sample information may be modified, or additional sample information can be entered manually in the dialog box fields. Click the "Add/Modify this Sample ID" when finished.*
4. Click the "Done" button once the data for all desired wells has been entered.

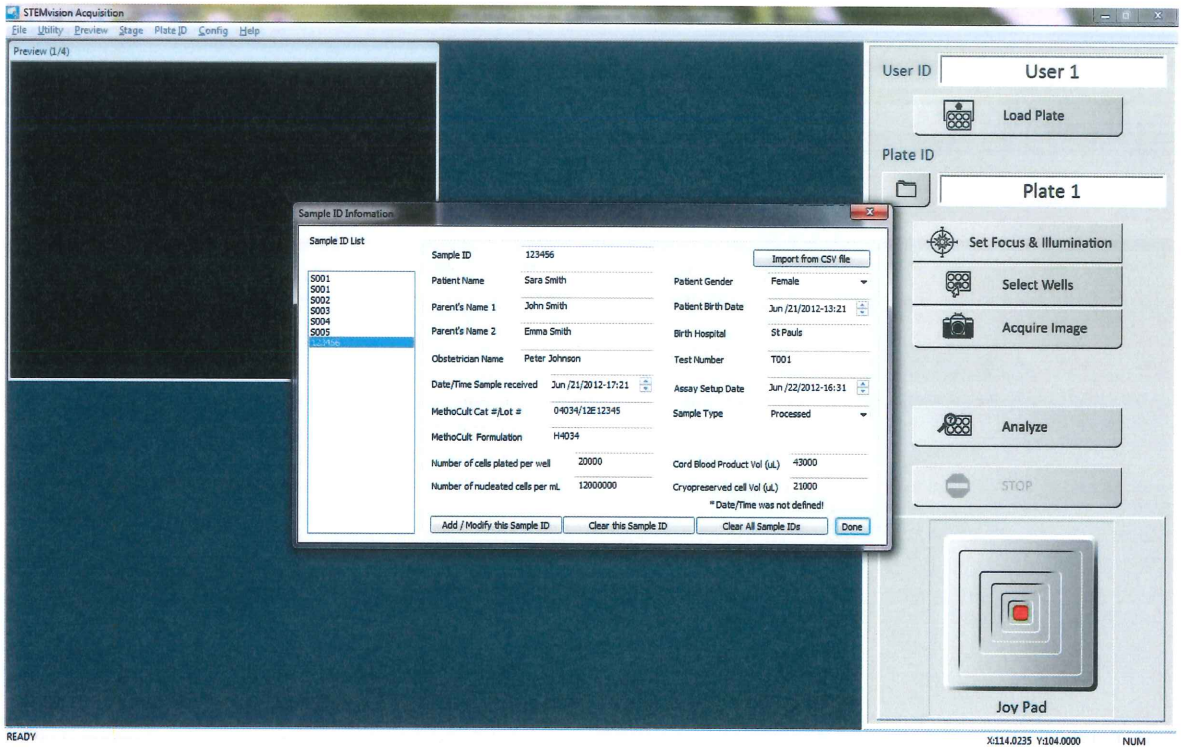



**Figure 6: Sample ID Information Dialog Box.**

## Manual Entry of Sample ID Information

1. Click on "Plate ID" in the Menu Bar and select "Sample ID Information".
2. Populate the fields of the "Sample ID Information" dialog box with the relevant information.
3. 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" (Figure 6).
4. Continue adding new Sample ID information as required.
5. Click the "Done" button when all samples to be imaged have been added to the list.

   *Note: Any Sample ID information added manually will be cleared on shut-down of the STEMvision™ Acquisition application. In order to save Sample ID Information, populate a CSV file as described under "Automatic Entry from a Pre-Populated CSV File".*

## Manual Definition of User Definable Fields (UDFs)

In addition to the CB specific sample information entered in the "Sample ID Information" dialog box (Figure 6), customized sample information can in entered in up to 4 UDFs.

1. Click on "Plate ID" in the Menu Bar and select "Define UDF Titles".
2. Enter the custom UDF names into the UDF fields.

   *Note: The customized UDF names will be set as defaults for future sessions but can be changed at any time. The UDF information can be found in the acquisition data log files (Section 6.6).*

### 4.4.3 Image Identification

To select the root folder where the acquired images, data log files (Section 6.6) and analysis files will be saved, click on the folder icon button in the Control Panel and select the root folder in the dialog box (Browse for Folder).

*Note: By default, images will be saved in the folder specified during the previous acquisition session.*

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 (e.g. Plate1), whereas the image file name corresponds to the Plate ID_Well ID.jpg (e.g. Plate1_A1.jpg).

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 (e.g. Plate1_A1_Sample1.jpg).

*Note: Once selected, "Include Sample ID in Image File Name" will be set as the default file naming convention for future acquisition but can be changed again at any time.*

Refer to Section 4.4.2 for more information on Plate ID, Well ID and Sample ID.

## 4.5 Setting Illumination and Focus

The STEMvision™ Acquisition application includes specific tools for properly setting the illumination and focus of the instrument. The focus values are a relative measure of contrast in the preview image. As the preview image becomes more focused, the relative contrast value will increase and reach a maximum at the optimum focus setting.

1. Note: When imaging multiple plates, the focus only needs to be set before the first plate is imaged. The focus should be set again before imaging additional plates if the instrument has been left unattended, or if the focus knob is disturbed accidentally. Click the "Set Focus & Illumination" button in the Control Panel.
2. Select a well by right-clicking on it in the Well Navigator dialog box.
3. Set the Illumination:
   i. Use the Joy Pad to find a location in the well that does not contain any colonies (i.e. only medium should be seen in the Preview Window) as shown in Figure 7. If an area with no colonies cannot be found, use the Joy Pad to find a location in the well that does not contain any large colonies or dark shadow areas (i.e. primarily medium should be seen in the Preview Window).
   ii. 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.

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

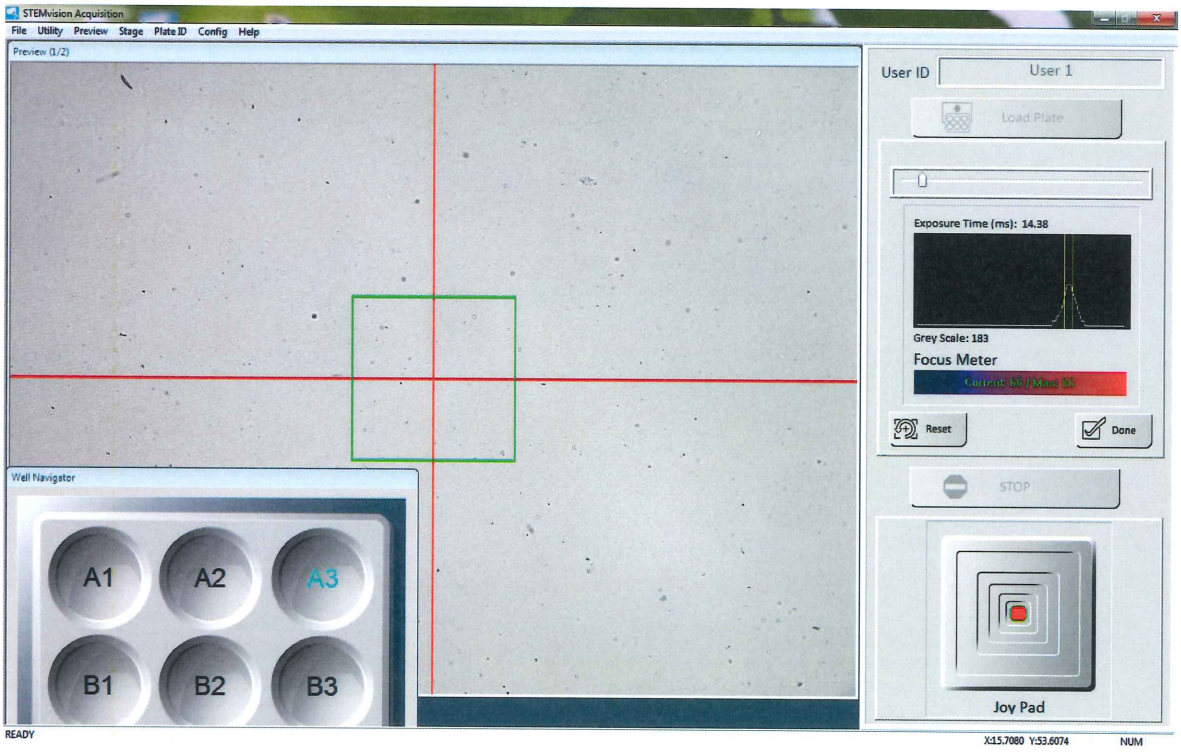



**Figure 7: Setting the Illumination in the "Set Focus and Illumination" Dialog Box of the STEMvision™ Acquisition Application.**

4. Set the Focus:
   i. Use the Joy Pad to find a location in the well so that a myeloid (i.e. CFU-GM) colony with individual cells around its periphery is placed inside the focus area (i.e. green box in the Preview Window) as shown in Figure 8.
   ii. Put the image in the Preview Window out of focus by turning the coarse focus knob (Figure 1D).
   iii. Turn the coarse knob and observe as the "Current" value approaches the "Max" value in the "Focus Meter". Continue until the "Current" value starts to decrease, relative to the "Max" value. The "Max" value should be >100. If the "Max" value is <100, use the Joy Pad to navigate to a larger myeloid colony and start the procedure for setting the focus again.

   *Note: The "Current" contrast value will increase as the image focus improves, and will reach a "Maximum" at a focus level optimal for STEMvision™ imaging. If the focus knob is turned beyond this optimal focal plane, the "Current" contrast value will begin to decrease below the established "Max" contrast value shown in the "Focus Meter".*
   iv. Turn the fine focus knob, in the opposite direction, (Figure 1E) so that the "Current" and "Max" values match as closely as possible (± *3 units*).

   *Note: Clicking the "Reset" button will set the "Max" contrast value to the "Current" value.*
   v. Click the "Done" button.

   *Note: After setting the focus, use the Joy Pad to pan over the well and confirm that most colonies appear in the plane of correct focus. If necessary, repeat the focus steps with a colony that better represents the average depth of colonies in the MethoCult™ medium.*

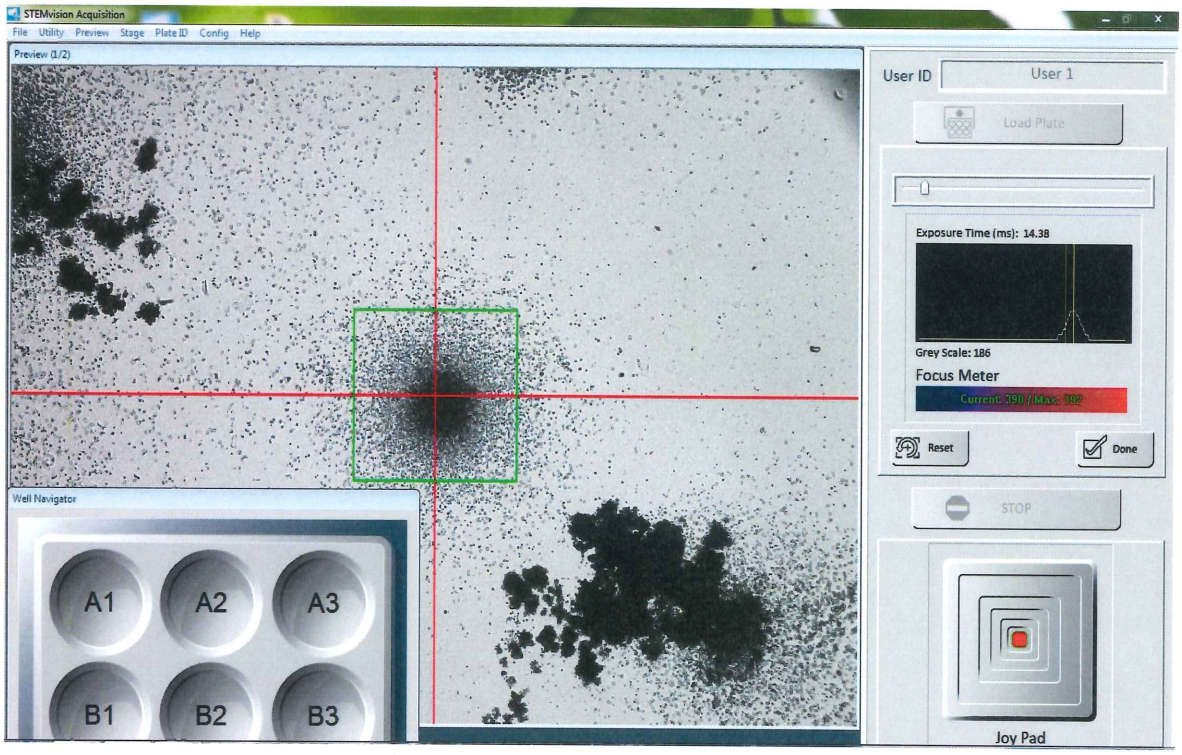



**Figure 8: Setting the Focus in the "Set Focus and Illumination" Dialog Box of the STEMvision™ Acquisition Application.**

## 4.6 Image Acquisition

The STEMvision™ Acquisition application produces a single composite image of each culture well that is comprised of 35 separate camera images. These composite image files are saved to the selected destination root folder (see Section 4.4.3).

***Important:*** *If printed "Parent" and "Lab" CFU assay report forms will be required, sample information must be entered in a CSV file and imported as described in Section 4.4.2 before image acquisition.*

### 4.6.1 Selecting Wells

Before images can be acquired, the wells to be imaged must be selected. Specific sample information may be entered for each well if desired.

*Note: If printed "Parent" and "Lab" CFU assay report forms will be required, a sample ID must be entered for each well to be imaged. Sample ID information must have been defined previously as described in Section 4.4.2.*

1. Click the "Select Wells" button in the Control Panel. A dialog box (Select Wells) 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 (Figure 9).
2. Select an individual well, or all wells that are replicates, by clicking on the 6-well plate graphic. As wells are selected, the matching lines on the "Well Info" table are highlighted.

   *Note: Click on "Select All" at the bottom left corner of the dialog box to select all wells of the 6-well plate.*
3. Select a previously defined Sample ID for the selected well(s) by using the drop-down menu.

   *Note: If the same hematopoietic cell sample was plated at different densities (i.e. number of cells/well) enter this information now, if it had not been previously done (see Section 4.4.2).*

   *Optional: Information can also be entered under each customized UDF field.*
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.
5. Repeat steps 2 - 4 until the data for all wells to be imaged has been entered in the "Well Info" table.
6. Click the "Done" button when finished.

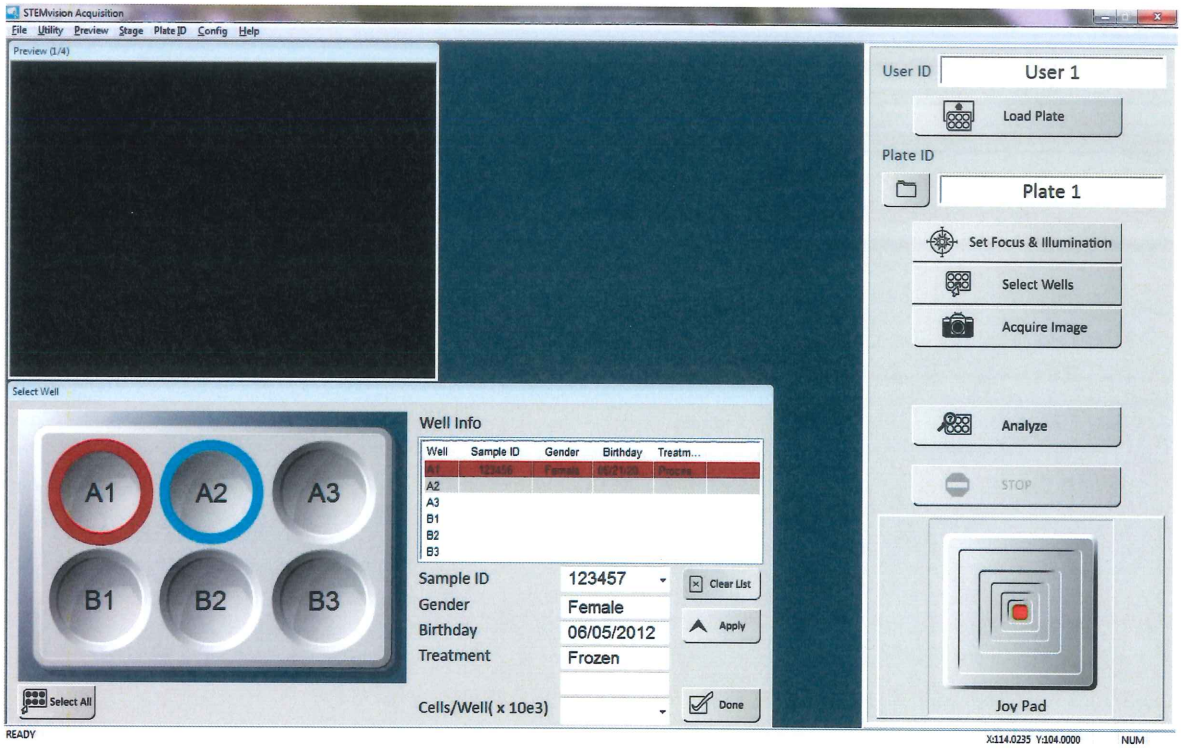



**Figure 9: Sample ID Information Dialog Box**

### 4.6.2 Acquiring Images

1. Click the "Acquire Image" button in the Control Panel. A real-time preview will show the composite well image being produced as each of the 35 image tiles is sequentially acquired.

   *Note: Image acquisition can be cancelled at any time by clicking the "Stop" button in the Control Panel. The current image will not be saved if acquisition is stopped before all of the 35 tile images are acquired.*

2. Open the STEMvision™ enclosure door and remove the SmartDish™ plate from the sample tray once image acquisition has been completed for all desired wells.

3. To image additional plates, load the next SmartDish™ and acquire images as described in steps 1 - 2.

   *Note: The illumination and focus do not have to be set again if multiple SmartDish™ plates are to be imaged in immediate succession.*

   *Optional: To immediately analyze the data after image acquisition, click the "Analyze" button in the Control Panel. This will directly launch the STEMvision™ Analyzer application and will populate the "Input Image" field (Figure 10) with the previously acquired images. To clear previously acquired images from the "Input Image" field, right-click the "Analyze" button in the Control Panel of the STEMvision™ Acquisition application before starting image acquisition. Follow the steps outlined in Section 5.0 to complete the image analysis.*

4. After images of all cultures have been acquired, shut down STEMvision™ as described in Section 3.3.

# 5.0 STEMvision™ Analyzer Application

The STEMvision™ Analyzer application is designed to analyze STEMvision™-acquired images offline and can be run independently of the STEMvision™ instrument. Different STEMvision™ Analysis Packages have been optimized to identify, classify and count hematopoietic colonies from specific tissue types. Therefore, the correct STEMvision™ Analysis Package must be used to analyze images of 14-day CFU assays of BM, MPB or CB cells, or 7-day CFU assays of CB cells. This is because hematopoietic colonies produced by even the same type of progenitor cell from different tissues can appear morphologically different. In addition, colonies generated in a 7-day CFU assay of CB cells, performed using MethoCult™ Express (see Table 1) will look different than colonies generated in a 14-day CFU assay of the same cells performed using MethoCult™ Optimum.

The different STEMvision™ Analysis Packages and the CFU sub-types that they score are summarized in Table 1. Briefly, the "Human BM 14-Day" package scores total CFUs, CFU-E, BFU-E, CFU-G/M/GM (all three types of uni- and bi-potent myeloid progenitors in a single category) and CFU-GEMM. The "Human MPB 14-Day" and "Human CB 14-Day" packages score total CFUs, BFU-E, CFU-G/M/GM, and CFU-GEMM. MPB and CB cells do not contain significant numbers of CFU-E so this progenitor is not scored in these tissues. Finally, the "Human CB 7-Day" package counts the total number of CFUs in a CB sample without distinction of the different CFU sub-types. This package is ideal for CB banks because it allows the number of viable progenitor cells in a CB unit to be measured one week faster than with a conventional 14-day CFU assay. The 7-day CB CFU assay can be sufficient in this setting because it is the total number of CFUs in a CB unit that correlates most strongly with successful engraftment following allogeneic CB transplantation.

*Note: Use of a particular STEMvision™ Analysis Package to analyze images of cultures that differ from that specified for each analysis package (i.e. different tissue type or day of assay incubation) will result in incorrect CFU assay results.*

## 5.1 User Interface Overview

Start the STEMvision™ Analyzer application on the computer (double-click the icon on the desktop). The user interface of the STEMvision™ Analyzer application is shown in Figure 10.

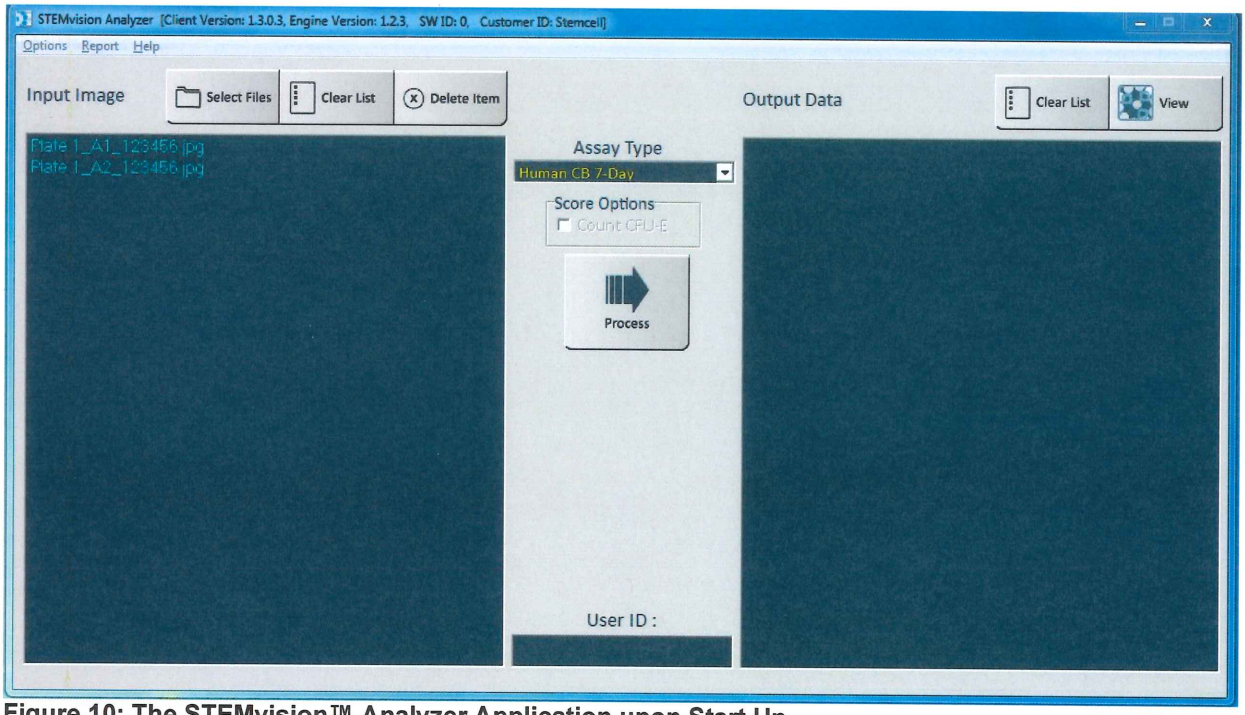



**Figure 10: The STEMvision™ Analyzer Application upon Start Up.**

- **Menu Bar**: Located along the top of the application window. It provides access to additional options such as printing "Lab" and "Parent" CFU assay report forms.
- **Assay Type**: Located in the center of the application window. This is a drop-down menu from which to specify the STEMvision™ Analysis Package to be used for image analysis.
- **Input Image**: Located on the left side of the application window. It shows the list of images that will be analyzed.
- **Output Data**: Located on the right side of the application window. It shows the list of images that have been analyzed.
- **User ID**: Located on the middle-bottom of the application. It identifies the operator for a given analysis session. If entered, the User ID information is recorded in the analyzer report file (Section 6.6).

## 5.2 Analysis Set Up

### 5.2.1 Generating CFU Assay Report Forms

In order to generate "Lab" and "Parent" CFU assay report forms, ensure that a CSV file is populated and imported into the STEMvision™ Acquisition application before acquiring images (see Section 4.4.2) and that a Sample ID has been defined at the time of acquisition (see Section 4.6.1).

1. Click on "Report" in the Menu Bar and select "Auto Print". This will automatically generate "Lab" and/or "Parent" CFU assay report forms once images have been analyzed.

   *Note: Once selected, "Auto Print" will be the default setting for future analysis.*

2. Customize options for generating CFU assay report forms. Ensure this is done before starting image analysis. Once the following options have been selected, they will be set to default for future analysis but can be changed again at any time. The following options are located in the Menu Bar under "Report":

   - **Institute Information:** Select "Report Information" and enter the desired information. To include a logo, click on "Select Logo File" in the dialog box and select the correct image file. The image file must be in "bmp" format. Click the "Done" button when finished.

     *Note: The "bmp" file will be re-sized to fit a resolution of 400 pixels in width. The aspect ratio of the file should be approximately 2:1 (W:H) or greater.*

   - **Obstetrician/Physician Information:** Select "Report Receiver Title" and choose either "Physician" or "Obstetrician". This will specify the type of medical practitioner next to their name in the reports.
   - **CC (Concurrent Copy) Information:** Select "Edit CC Name" and enter the name(s) of person(s) that should receive a duplicate copy of the CFU assay report form.

     *Note: CFU assay report forms will not automatically be sent to the specified individuals.*

   - **Output Format:** Select "Output Format" and choose either "To Printer" for direct printing of the document, or "To PDF" to save an electronic pdf format copy of the report.
   - **Paper Format:** Select "Paper Format" and choose either "A4" or "Letter" format.
   - **"Lab" or "Parent" CFU Assay Report Forms:** Select "Report Type" and choose either "Lab", "Both" or "Parent". The "Lab" option can be selected for all types of the different CFU assays performed by STEMvision™. The "Both" and "Parent" options can only be used for the STEMvision™ Human Cord Blood 7-Day CFU Analysis Package or the STEMvision™ Human Cord Blood 14-Day CFU Analysis Package because the "Parent" form is designed for clients of a CB bank.
   - **PDF Output Folder:** Select "PDF Output Folder" and then the file destination where you want to save the pdf version of the reports. When generating both CFU assay report forms (i.e. "Lab" and "Parent") they will be saved as a single pdf file. The pdf file name for these reports corresponds to: Sample ID_cells plated per well ($x10^3$)_Plate ID_date and time of analysis _Assay Type.pdf. For example, file name: 123456_20.000_Plate 1_201212111533_hCB14Day corresponds to human CB sample 123456 plated at 20 x $10^3$ cells/dish in SmartDish™ plate 1, analyzed at 3:33 PM on December 11, 2012 using the STEMvision™ Human Cord Blood 14-Day CFU Analysis Package.

### 5.2.2 Generating Preview Result Images

To obtain a preview 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 (i.e. the Plate ID folder) and will be smaller in size as compared to the final result image.

*Note: Once selected, "Result Image" will be set to default for future analysis but can be changed again at any time.*

## 5.3 Image Analysis

### 5.3.1 Selecting Images for Analysis

Images can be selected for analysis by either using the STEMvision™ Analyzer application or Microsoft® Windows® file manager as follows:

- **STEMvision™ Analyzer application:** Click on the "Select Files" button (top left side of the application window) and then browse and select the sub-folder that contains the images to be analyzed (i.e. Plate ID folder). All STEMvision™-acquired image files in the selected folder will appear under the "Input Image" field (i.e. Plate ID_Well ID_Sample_ID.jpg). Repeat to add additional images from other plates to the "Input Image" list to be analyzed.
- **Microsoft® Windows® file manager:** Open a file manager window and browse to the sub-folder that contains the images to be analyzed (i.e. Plate ID folder). Select the images to be analyzed and then drag and drop them directly into the "Input Image" field of the STEMvision™ Analyzer application.

  *Note: Multiple images can be selected by holding the "Ctrl" key while selecting the files.*

  To select images from multiple plates or to search for specific criteria, open a file manager window and browse to the root folder for the image acquisition session to be analyzed. In the search box at the top right of the file manager window, type the search criteria of interest. For example, to list the images for all the wells acquired within a particular session, enter "*.jpg" into the search field.

  To analyze all the wells acquired for that session, select an individual image in the search results and press "Ctrl+A" to select all the image files. Drag the selected files to the "Input Image" box in the STEMvision™ Analyzer application. Similarly, more specific search criteria can be used to select images acquired on certain dates or with common Sample IDs or Plate IDs.

Image files can be removed from the "Input Image" list by selecting the file and clicking the "Delete Item" button (top left of the application window). Multiple files can be selected by pressing the "Ctrl" or "Shift" key while selecting the individual image files. To delete all of the image files, click the "Clear List" button (top left of the application window).

### 5.3.2 Analyzing STEMvision™ Acquired Images

1. Select the appropriate "Assay Type" corresponding to the tissue (BM, MPB or CB), day of scoring (Day-7 or Day-14), and assay condition (with or without EPO) from the drop-down menu. Refer to Table 1 to determine which STEMvision™ Analysis Package to use for your application.

   *Note: When using the STEMvision™ Human Bone Marrow 14-Day CFU Analysis Package, counting of CFU-E-derived colonies is optional. To include CFU-E in the analysis results, check the "Count CFU-E" box located in the "Score Option" field.*

2. Click the "Process" button to start data analysis. A progress window will show the images being analyzed and their progress (Figure 12).

   *Note: Image analysis can be cancelled at any time by clicking the "Abort" button in the dialog box. Any image that has not been fully analyzed will be removed from the "Input Image" field and will be added to the "Output Data" field with the prefix "User Abort". No result files will be generated for these images.*

3. As the images are analyzed, the file names will move from the "Input Image" to the "Output Data" field (Figure 12). The analysis result files will be saved in the same sub-folder as the images (i.e. Plate ID folder).

   *Note: For images that have been successfully analyzed, the file name in the "Output Data" box will be identical to the "Input Data" file name with an addition "_Assay Type_Result.txt" at the end (e.g. Plate ID_Well ID_Sample ID_hCBDay14_Result.txt).*

4. Click the "Clear List" button (Top right side of the application window), to clear the list of analyzed images.

   *Note: This will remove files from the "Output Data" list only, and will have no effect on the resultant data files that are stored in the image sub-folder (i.e. Plate ID folder). The data files will remain stored in their original location.*

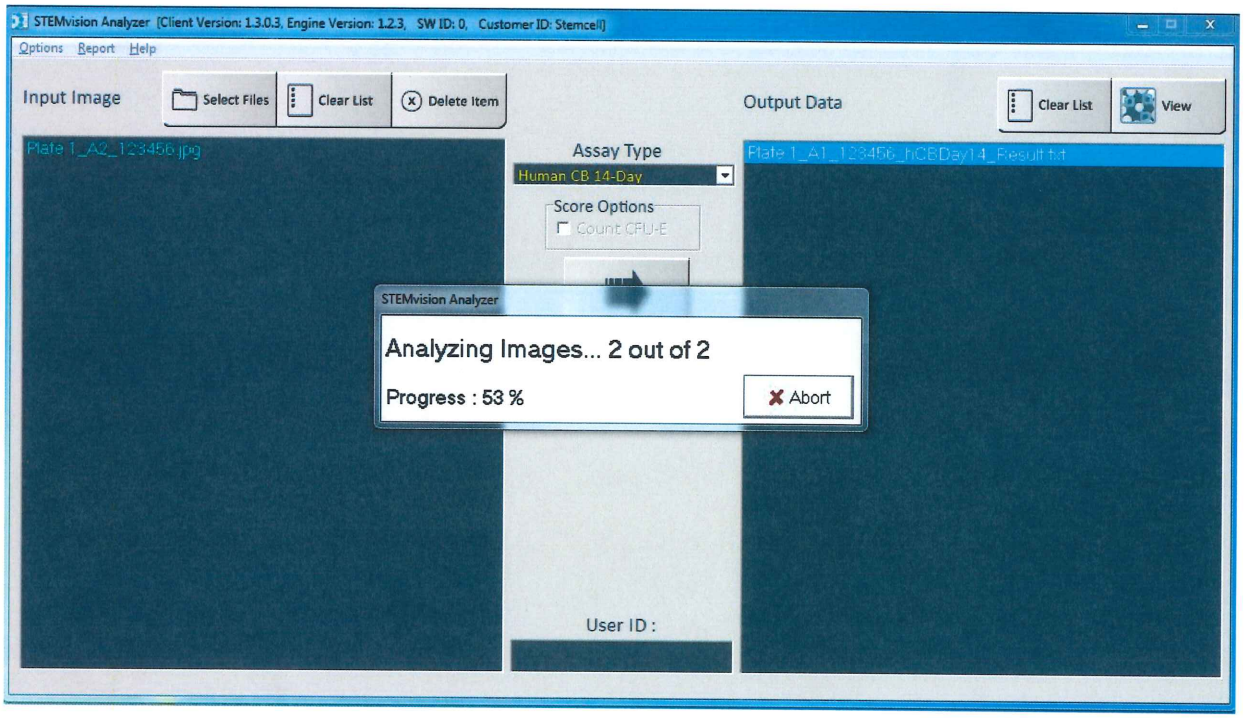



**Figure 11: STEMvision™ Analyzer Indicating Progress.**

5. View analyzed images:
   - For a quick (read-only) view of the analyzed images, select a file in the "Output Data" field and click "View". This will open the analyzed image in the STEMvision™ Colony Marker Application and show a tabulation of the analysis results along with the annotated image with the different sub-types of hematopoietic colonies identified by colored circles.
   - For further details on how to view results, see Section 6.2.2 and Section 6.3.

# 6.0 Viewing Results

Results from STEMvision™ image analysis can be viewed in several different ways:

- "Lab" and "Parent" CFU assay report forms can be generated for samples that have a Sample ID associated to them at the time of image acquisition (Section 6.1).
- The STEMvision™ Colony Marker application can be used to view results of an individual culture image (Section 6.2).
- Microsoft® Excel® can be used to import a summary table of colony counts (Section 6.3).
- Log files can be used to view additional results (Section 6.6).

## 6.1 Viewing CFU Assay Report Forms

The results of 7-Day and 14-Day CFU assays generated by STEMvision™ can be printed out in two different report formats: The "Lab" CFU assay report form contains detailed information on the CFU assay for the laboratory or transplant physician. A second "Parent" CFU assay report form is suitable for presentation of CB sample CFU assay results to parents banking their child's CB with a private CB bank. Refer to Table 1 for an overview of the types of reports that can be produced for each hematopoietic tissue type.

If "Auto Print" was selected at the time of analysis, "Lab" and/or "Parent" CFU assay report forms will be automatically generated after image analysis and will be based on the options set before image acquisition (Section 5.2.1). See Appendix 1 for examples of "Lab" and "Parent" CFU assay report forms.

To generate a CFU assay report from previously analyzed images at a later time:

1. In the STEMvision™ Analyzer application, select the appropriate "Assay Type" corresponding to the tissue (BM, MPB or CB), day of scoring (Day-7 or Day-14), and assay condition (with or without EPO) from the drop-down menu.
2. Click on "Report" in the Menu Bar of the STEMvision™ Analyzer application and select "Find".
3. Enter the Sample ID in the search field of the dialog box (STEMvision Report Publisher).

   *Note: The entire Sample ID name does not need to be entered. Searching can be done by using the first few characters of the Sample ID.*
4. Click the "Find" button.
5. Click on either the "Preview" button for a quick view of the report form, the "PDF" button to save a PDF version of the report form, or the "Print" button to directly print the report form (on the default printer selected in Windows®).
6. Click the "Done" button once finished.

*Note: Reports can only be generated if Sample ID information has been defined at the time of image acquisition.*

## 6.2 Viewing Individual Images

### 6.2.1 Read-Only View

A read-only view of the analyzed culture images can be accessed via the STEMvision™ Analyzer application following completion of the image analysis process (see last step in Section 5.3.2).

*Note: To edit the results, the image (jpg) and analysis (txt) files must be opened with the STEMvision™ Colony Marker application (see Section 6.2.2).*

### 6.2.2 Using the STEMvision™ Colony Marker Application

The STEMvision™ Colony Marker application is designed for:

- Viewing STEMvision™-acquired images.
- Manual scoring of colonies from images if desired.
- Viewing and editing results produced by the STEMvision™ Analysis Packages.

The STEMvision™ Colony Marker application can be used independently of the STEMvision™ instrument. Multiple images cannot be viewed at one time using this application.

Start the STEMvision™ Colony Marker application on the computer (double-click the icon on desktop). The user interface of the STEMvision™ Colony Marker application is shown in Figure 12.

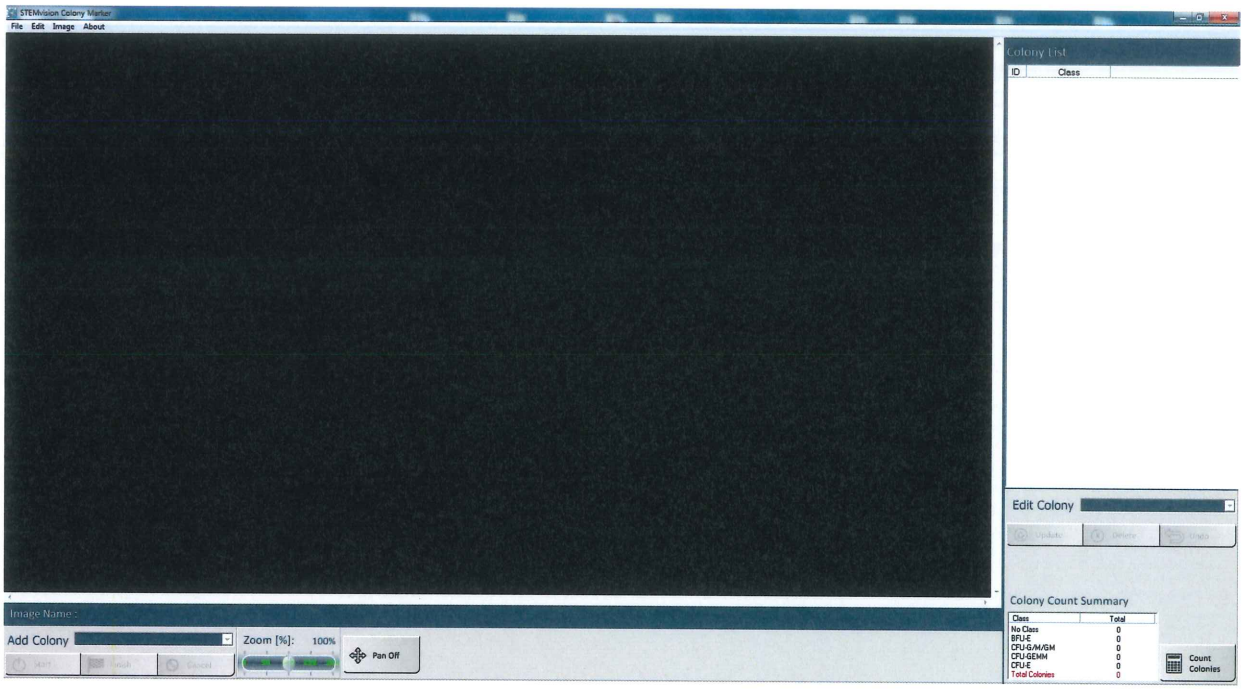



**Figure 12: The STEMvision™ Colony Marker Application upon Start Up.**

- **Menu Bar:** Located along the top of the application window.
- **View Window:** Located below the Menu Bar. The loaded image will be displayed here along with the analysis results (if loaded) as colored circles surrounding each individual colony. The colony ID number is shown near the top left of the colored circle surrounding each colony.
- **Colony List:** Located on the right side of the application window. Gives a tabular summary of each colony, indicating its ID number so that it can be identified in the image, and the CFU sub-type that it has been scored as by STEMvision™ (i.e. CFU-E, BFU-E, CFU-G/M/GM or CFU-GEMM).
- **Edit Colony:** Located on the right side of the application window (grey area). Allows editing of analyzed images.
- **Colony Count Summary:** Located on the bottom right corner of the application window. Summarizes the numbers of different types of colonies in the analyzed culture.
- **Count Colonies Button:** Located on the bottom right corner of the application window (beside the Colony Count Summary table). Clicking this button will update the Colony Count Summary table after analysis results are loaded or edited.
- **Add Colony:** Located on the bottom left corner of the application window. Allows new colonies to be added to the analyzed image.
- **Zoom Control Bar:** Located on the bottom left of the application window. Changes image magnification.
- **Pan Off/On Button:** Located on the bottom left of the application window. "Pan On" (hand cursor) allows image navigation by clicking and dragging the image. "Pan Off" (arrow cursor) locks the image in a specific position and allows selection of colonies.

1. Click on "File" in the Menu Bar and select "Open Image". Browse through the folders and select the image you want to view. This will open up the acquired image only (i.e. the different colony types will not be shown).

   *Note: Images may be dragged and dropped directly from a file manager window into the preview window.*

2. Click on "File" in the Menu Bar and select "Open Data". Browse and select the data (txt) file generated during the image analysis (i.e. ImageName_AssayType_Result.txt).

   *Note: Data may be dragged and dropped directly from a file manager window into the "Colony List" field.*

   Colonies will be marked on the image as shown in Figure 13. The colored circles around each colony correspond to the CFU sub-type that has been scored by STEMvision™:

   - Orange: CFU-E
   - Red: BFU-E
   - Yellow: CFU-G, CFU-M or CFU-GM
   - Blue: CFU-GEMM
   - Green: No Class (i.e. used in the STEMvision™ Human Cord Blood 7-Day CFU Analysis Package that counts total CFUs only)

At any time the zoom level of the image can be adjusted by sliding the Zoom Control Bar or by rolling the mouse wheel button up or down.

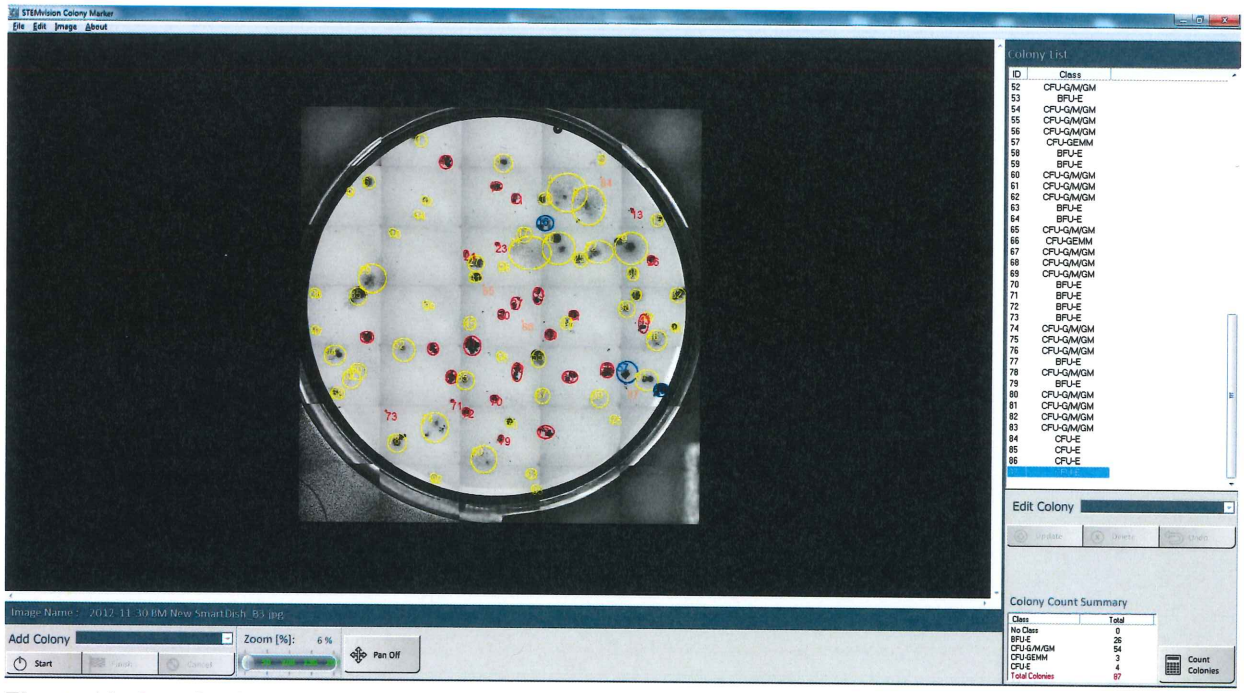



**Figure 13: Acquired Image and Analysis Data Files Opened in the STEMvision™ Colony Marker Application.** This image corresponds to a 14-Day BM CFU assay culture analyzed with the STEMvision™ Human Bone Marrow 14-Day CFU Analysis Package.

## 6.3 Creating a Summary Table of Colony Counts in Microsoft® Excel®

The results of STEMvision™ analysis of multiple cultures can be tabulated in a Microsoft® Excel® spreadsheet without viewing the images individually. A summary table as shown in Figure 14 will be generated.

| Image Name | Total-E | Total-M | Total | CFU-GEMM | CFU-GM | BFU-E | CFU-E | Errors |
|---|---|---|---|---|---|---|---|---|
| BM27_B2_hCBDay14_Result.txt | 43 | 79 | 122 | 8 | 71 | 30 | 13 | Colony number above target range; |

**Figure 14: Example of a Summary Table Generated in Microsoft® Excel®.**

The name(s) of the analyzed image(s) are shown in the first column, and the counts for each colony type are shown in the subsequent columns. "Total-E" refers to the total number of colonies of the erythroid lineage (i.e. colonies produced by BFU-E plus CFU-E, if applicable). "Total-M" refers to total number of colonies of the myeloid lineage (i.e. colonies produced by CFU-G, CFU-M, CFU-GM plus CFU-GEMM). The column labeled "Total" indicates the sum of all colonies identified in the image.

If errors are detected in the image during analysis, these will be described in the last column. Note that error messages reflect technical problems with CFU assay set-up or image acquisition. They are not related to problems with the STEMvision™ instrument. Possible errors include having either too many or too few colonies per dish than is recommended for optimal quantitation of CFU numbers in the sample (i.e. due to over- or under-plating of the sample), or problems with image quality (e.g. due to incorrect operator setting of exposure, the presence of condensation on the SmartDish™ lid, or excessive shadow areas possibly due to uneven dispersion of MethoCult™ medium or culture dehydration). Multiple error messages will be separated by a semicolon.

CFU assay Result Tables can be compiled for individual plates, individually selected wells, or based on specific search criteria.

*Note: Any existing content in the Microsoft® Excel® spreadsheet that overlaps with the resulting table will be deleted.*

1. Individual plates: From within a Microsoft® Excel® spreadsheet, right-click on the cell where the Result Table is to be inserted and select "STEMvision: Select results folder" from the drop-down menu. Browse to and select the image sub-folder (i.e. Plate ID folder) that contains the analysis result files.

   *Note: For images analyzed with the STEMvision™ Human Cord Blood 7-Day CFU Analysis Package that does not score the CFU sub-types, values will be shown in the "Total" colony counts column only.*

2. Individual wells: From within a Microsoft® Excel® spreadsheet, right-click on a cell where the Result Table is to be inserted and select "STEMvision: Select results files" from the drop-down menu. Browse to and select the image sub-folder (i.e. Plate ID folder) that contains the analysis result files. Select the results files to be included in the summary table (multiple files may be selected by holding the "Ctrl" or "Shift" keys while selecting). Click the "Open" button to compile the summary table for the selected results.

3. Based on specific search criteria: From within a Microsoft® Excel® spreadsheet, right-click on a cell where the Result Table is to be inserted and select "STEMvision: Select results files" from the drop-down menu. Browse to the root folder that contains the results files. In the search box at the top right of the file dialog, type the search criteria of interest (use '*' for wild card searches). For example, to list all the results for images analyzed with the STEMvision™ Human Cord Blood 14-Day CFU Analysis Package, you may enter "*hCBDay14*" into the search field. All results files that match the search criteria will be displayed in the dialog box. To generate a summary table for all the results matching the search criteria,

select an individual file in the search results and press "Ctrl+A" to select all the files. Alternately, multiple individual results files may be selected by holding the "Ctrl" or "Shift" keys while selecting. Click the "Open" button to compile the summary table for the selected results.

## 6.4 Editing Results

The CFU assay scoring results produced by the STEMvision™ Analysis application can be changed for individual colonies if desired using the STEMvision™ Colony Marker application.

*Note: Any editing will not be reflected when generating the "Lab" or "Parent" CFU assay report forms.*

1. Open a STEMvision™-acquired image and its corresponding data (txt) file in the STEMvision™ Colony Marker application as described in Section 6.2.2.
2. To add a new colony that was not previously counted:
   i. Click the "Start" button in the Add Colony field. A brown circle, with its ID number, will appear in the middle of the image.
   ii. Click and drag the brown circle over the new colony to be marked. Adjust the size of the outline as necessary by clicking and dragging the white handles (squares).
   iii. Choose the colony sub-class that corresponds to the new colony by selecting it in the Add Colony drop-down menu.

   *Note: To cancel addition of a new colony, click the "Cancel" button in the Add Colony field at any time.*

   iv. Click the "Finish" button in the Add Colony field. The colony will be added to the image and in the Colony List.
   v. Repeat steps i - iv until all the additional colonies have been added.
   vi. Click the "Count Colonies" button in the Colony Count Summary field to update the number of colonies in the Colony Count Summary table.
3. To change the position or size of colony outlines:
   i. Select the colony by clicking on it in the image or selecting its colony ID in the Colony List field.
   ii. Drag the white handles (squares) to adjust the size of the colony outline or drag the entire outline to a new position. Once the outline shape or position has been changed, the outline color will change to brown.
   iii. Click the "Update" button in the Edit Colony field to finalize the change. This will update the color of the outline to its original color.

   *Note: To cancel the edits, click the "Undo" button below the Edit Colony field.*

   vii. Repeat steps i - iii until all necessary changes have been completed.
4. To change the CFU sub-type of a colony:
   i. Select the colony by clicking on it in the image or selecting its colony ID in the Colony List field.
   ii. Select the new colony sub-class from the Edit Colony drop down menu. The outline color will change to brown indicating that a change has been made.
   iii. Click the "Update" button in the Edit Colony controls to finalize the change. The color of the outline will be automatically updated to that of the newly specified CFU sub-type.

   *Note: To cancel the edits made to the colony outline, click the "Undo" button below the Edit Colony field.*

   iv. Repeat steps i - iii until all necessary changes have been completed.
   v. Click on the "Count Colonies" button in the Colony Count Summary field to update the number of colonies in the Colony Count Summary table.

5. To delete a counted colony:
   i. Select the colony by clicking on it in the image or selecting its colony ID in the Colony List field.
   ii. Click the "Delete" button in the Edit Colony field.
   iii. Repeat steps i. and ii. until all the desired deletions have been made.
   iv. Click the "Count Colonies" button in the Colony Count Summary field to update the number of colonies in the Colony Count Summary table.

   *Note: It is recommended that the updated data file be saved as a separate file from the original produced automatically by the STEMvision™ Analysis application. Click on "File" in the Menu Bar and select "Save Data." A prompt for a file name and destination will appear. If manually edited results are to be included in summary tables compiled in Microsoft® Excel®, the results file name must include the following: "Mod_".*

## 6.5 Manually Scoring an Acquired Image

Images acquired using STEMvision™ can be scored manually using the STEMvision™ Colony Marker application.

1. Open the application and load the desired image file as described in Section 6.2.2. There is no need to open the results file as well.
2. Click on "Edit" in the Menu Bar and select "Mark Colonies".
3. Mark the desired colonies as described in Section 6.4.
4. Save the results by clicking on "File" in the Menu Bar and select "Save Data." A prompt for a file name and destination folder will pop up. The data is saved in the form of a .txt file.

*Note: If manually scored results are to be included in summary tables compiled in Microsoft® Excel®, the results file name must include the word "Manual".*

## 6.6 Acquisition and Analyzer Logs

Information for each image acquisition and analysis session is logged by STEMvision™. These logs contain information related to the Sample ID, imaging setup and analysis information. This information may be accessed via an "Info" file.

Acquisition logs capture the following information related to the acquisition: STEMvision™ unit ID, Plate ID, barcode number, User ID, UDFs for each well, links to the original image files and a report file describing the STEMvision™ instrument settings during the acquisition (exposure level and time, focus contrast, and camera settings).

Analysis logs capture the following information related to the analysis: Plate ID, assay type, analysis time, User ID, and links to each analysis results file.

To view the logged information, browse to the image sub-folder (i.e. Plate ID folder) in the root folder. Within this folder, locate the file named "Plate ID_Info.htm" ("Plate ID" being the name entered for the plate during image acquisition). Open the file to display the acquisition and analysis information for the plate.

# 7.0 Care and Maintenance

STEMvision™ should not come into direct contact with any biological materials. Adhere to good laboratory practices as specified by your institution when using STEMvision™.

## 7.1 Normal Maintenance

Turn OFF the STEMvision™ instrument when it will not be used overnight or for a significant period of time during the day (see Section 3.3). This may help to extend its lifetime. As STEMvision™ is an enclosed system, it is sufficient to wipe the outside of the instrument occasionally with an appropriate non-corrosive decontaminant solution to keep it clean. If dilute bleach is used, wipe clean with water afterwards.

Adhere to the "Optimal Operating Conditions" specified in Section 8.0 to increase the lifetime of the STEMvision™ instrument.

## 7.2 Verification of Stage Positioning

The correct positioning of STEMvision™'s moveable stage can be verified using the STEMvision™ calibration plate provided with each system.

1. Place the calibration plate into the stage insert, ensuring the plate is firmly seated and flush against the bottom right corner of the stage insert.
2. Close the enclosure door and start the STEMvision™ Acquisition application (see Section 4.1).
3. Click the "Set Illumination & Focus" button in the Control Panel and right-click on well B3 in the Well Navigator dialog box to move the stage to the center of this well. A cross-shaped slot should appear in the preview window. If necessary, turn the focus knobs to bring the edges of the cross into sharp focus.
4. Confirm that the intersection of the red grid lines falls within the center area of the cross (i.e. open space outlined in blue dashed square), as shown in Figure 15 (left image).
5. Right-click on well A1 in the Well Navigator dialog box to move the stage to the center of this well. A cross-shaped slot should again be visible in the preview image. Confirm again that the red grid lines fall within the center area of the cross, as shown in Figure 15 (left image).
6. If the red grid lines fall outside of the boundaries of the cross shape for either wells A1 or B3, then the stage is not calibrated correctly (Figure 15, right image).

*Note: If the stage calibration is determined to be incorrect, contact STEMCELL Technologies so that this can be corrected by a qualified individual.*

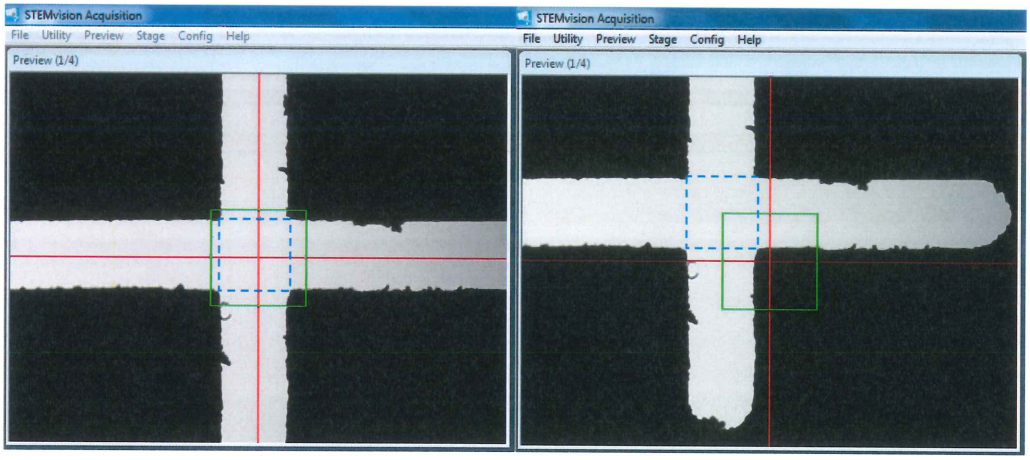



**Figure 15: STEMvision™ Stage Position: Correct (left) and Incorrect (right) Positioning.** Blue dashed square indicates the targeted area (center of cross).

## 7.3 Cleaning an External Spill

To clean a spill on the outside of STEMvision™, unplug the instrument and use an appropriate decontaminant solution to wipe with a soft, clean paper towel. If dilute bleach is used, wipe clean with water afterwards.

## 7.4 Cleaning an Internal Spill

To clean a spill inside STEMvision™, unplug the instrument, and open either the enclosure door (Figure 1F] or lens access door [Figure 1A]) as necessary. Use an appropriate decontaminant solution to wipe with a soft, clean paper towel. If dilute bleach is used, wipe clean with water afterwards. Take extreme care not to scratch the camera lens or dislodge any internal parts.

If spills inside the STEMvision™ instrument cannot be reached by opening either of the above access doors, contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com with a description of the failure so the appropriate corrective action can be determined.

## 7.5 Malfunction

In the event of a malfunction, turn OFF STEMvision™ using the switch located on the back of the instrument (Figure 2G). Contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com with a description of the failure so the appropriate corrective action can be determined.

# 8.0 Specifications

| | |
|---|---|
| Capacity | One 6-well SmartDish™ plate at a time |
| Dimensions | 478 mm W x 335 mm D x 347 mm H<br>18.82 in W x 13.19 in D x 13.66 in H |
| Weight | STEMvision™: 59 lbs or 27 kg<br>Computer: 28 lbs or 12 kg |
| Power Requirements | 120V/240V, 1.5A, 60Hz/50Hz 1Ph<br>Fuse F 2A L 250VAC |
| Optimal Operating Conditions | 15 - 30°C<br>20 - 85% relative humidity<br>Not specified for use inside an incubator<br>Does not require placement in a biohazard safety cabinet<br>Indoor use only<br>Not to be used in a cold room |
| Storage and Shipping Conditions | -30°C to 50°C<br>10 - 90% relative humidity |
| Applied Standards | Certified to CAN/CSA STD 22.2 No. 61010-1-12<br>Conforms to UL STD 61010-1:2012<br>Conforms to UL STD 61010-1:2004<br>Conforms to EN STD 61010-1:2010<br>Conforms to IEC STD 61326-1:2005 / EN STD 61326-1:2006<br>FCC Part 15 Subpart B: 2012<br>ICES-003:2012 |
| Meets all the Provisions of the Indicated EC Directives | 2006/95/EC (low-voltage directive) and 2004/108/EC |

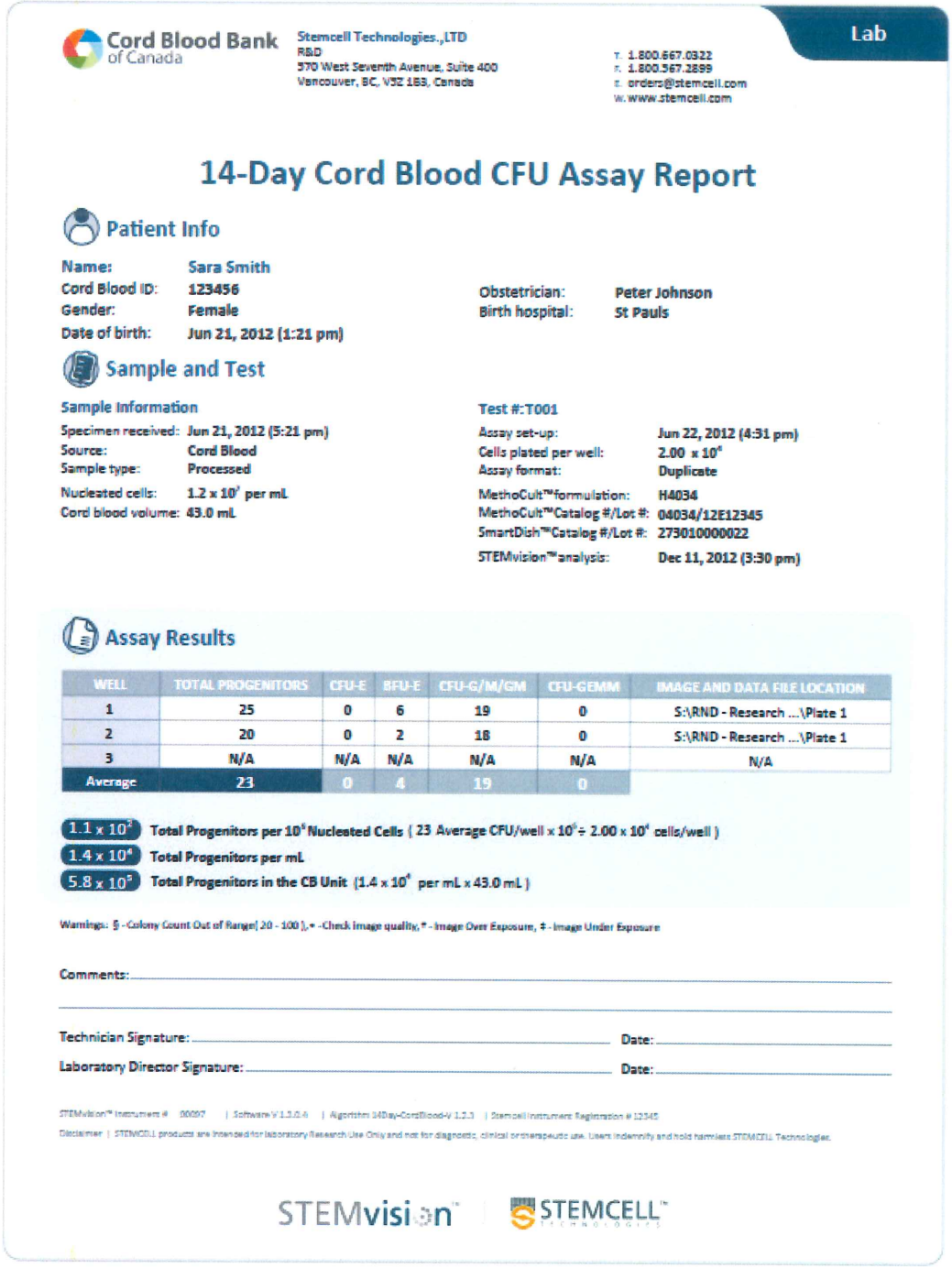
STEMvision™ is designed and manufactured so that it does not endanger the safety of operators when properly installed, maintained and used in applications for which it was intended. STEMvision™ meets the requirements of EC directives 2006/95/EC and 2004/108/EC. STEMvision™ meets the requirements of CAN/CSA-C22.2 No. 61010.1-12 and UL standard No. 61010-1 (safety requirements for electrical equipment for measurement, control and laboratory use).

If STEMvision™ is retired from regular use, return it to the shipping crate and store in conditions as specified.

The STEMvision™ unit has the following symbol affixed to the rear: 

This symbol denotes that the device should not be disposed of in municipal waste collection centers. STEMvision™ has been designed for easy dismantling and recycling. Contact STEMCELL Technologies regarding end of life care for STEMvision™.

# Appendix 1: Examples of "Lab" and "Parent" CFU Assay Report Forms



Cord Blood Bank of Canada

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Lab

## 14-Day Cord Blood CFU Assay Report

### Patient Info

Name: Sara Smith
Cord Blood ID: 123456
Gender: Female
Date of birth: Jun 21, 2012 (1:21 pm)

Obstetrician: Peter Johnson
Birth hospital: St Pauls

### Sample and Test

**Sample Information**

Specimen received: Jun 21, 2012 (5:21 pm)
Source: Cord Blood
Sample type: Processed
Nucleated cells: 1.2 x $10^7$ per mL
Cord blood volume: 43.0 mL

**Test #:T001**

Assay set-up: Jun 22, 2012 (4:31 pm)
Cells plated per well: 2.00 x $10^4$
Assay format: Duplicate
MethoCult™formulation: H4034
MethoCult™Catalog #/Lot #: 04034/12E12345
SmartDish™Catalog #/Lot #: 273010000022
STEMvision™analysis: Dec 11, 2012 (3:30 pm)

### Assay Results

| WELL | TOTAL PROGENITORS | CFU-E | BFU-E | CFU-G/M/GM | CFU-GEMM | IMAGE AND DATA FILE LOCATION |
|---|---|---|---|---|---|---|
| 1 | 25 | 0 | 6 | 19 | 0 | S:\RND - Research ...\Plate 1 |
| 2 | 20 | 0 | 2 | 18 | 0 | S:\RND - Research ...\Plate 1 |
| 3 | N/A | N/A | N/A | N/A | N/A | N/A |
| Average | 23 | 0 | 4 | 19 | 0 | |

1.1 x $10^2$ Total Progenitors per $10^4$ Nucleated Cells ( 23 Average CFU/well x $10^4$ ÷ 2.00 x $10^4$ cells/well )
1.4 x $10^4$ Total Progenitors per mL
5.8 x $10^7$ Total Progenitors in the CB Unit (1.4 x $10^4$ per mL x 43.0 mL )

Warnings: § - Colony Count Out of Range( 20 - 100 ), ∞ - Check image quality, * - Image Over Exposure, ‡ - Image Under Exposure

Comments: _____

Technician Signature: _____ Date: _____

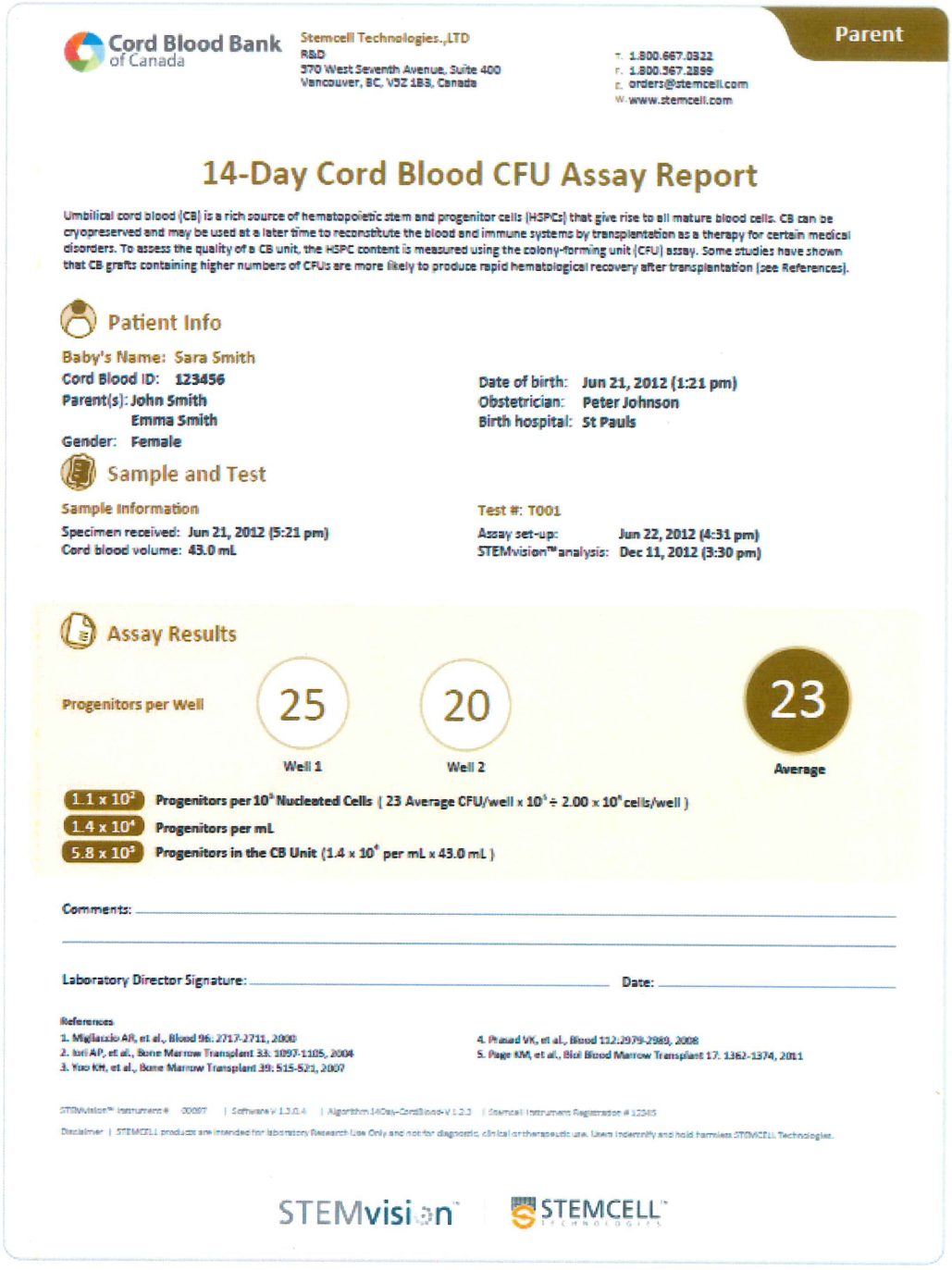
Laboratory Director Signature: _____ Date: _____

STEMvision™ Instrument # 00097 | Software V 1.3.0.4 | Algorithm 14Day-CordBlood-V 1.2.3 | Stemcell Instrument Registration # 12345

Disclaimer | STEMCELL products are intended for laboratory Research Use Only and not for diagnostic, clinical or therapeutic use. Users indemnify and hold harmless STEMCELL Technologies.

STEMvision™ STEMCELL™ TECHNOLOGIES

**Figure 16: "Lab" Format of the CFU Assay Report Form for CB Cells.**



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Parent

# 14-Day Cord Blood CFU Assay Report

Umbilical cord blood (CB) is a rich source of hematopoietic stem and progenitor cells (HSPCs) that give rise to all mature blood cells. CB can be cryopreserved and may be used at a later time to reconstitute the blood and immune systems by transplantation as a therapy for certain medical disorders. To assess the quality of a CB unit, the HSPC content is measured using the colony-forming unit (CFU) assay. Some studies have shown that CB grafts containing higher numbers of CFUs are more likely to produce rapid hematological recovery after transplantation (see References).

## Patient Info

Baby's Name: Sara Smith
Cord Blood ID: 123456
Parent(s): John Smith
Emma Smith
Gender: Female

Date of birth: Jun 21, 2012 (1:21 pm)
Obstetrician: Peter Johnson
Birth hospital: St Pauls

## Sample and Test

Sample Information
Specimen received: Jun 21, 2012 (5:21 pm)
Cord blood volume: 43.0 mL

Test #: T001
Assay set-up: Jun 22, 2012 (4:31 pm)
STEMvision™ analysis: Dec 11, 2012 (3:30 pm)

## Assay Results

Progenitors per Well

| Well 1 | Well 2 | Average |
|---|---|---|
| 25 | 20 | 23 |

$1.1 \times 10^2$ Progenitors per $10^5$ Nucleated Cells ( 23 Average CFU/well x $10^5$ ÷ 2.00 x $10^6$ cells/well )

$1.4 \times 10^4$ Progenitors per mL

$5.8 \times 10^2$ Progenitors in the CB Unit (1.4 x $10^4$ per mL x 43.0 mL )

Comments: _____

_____

Laboratory Director Signature: _____ Date: _____

References

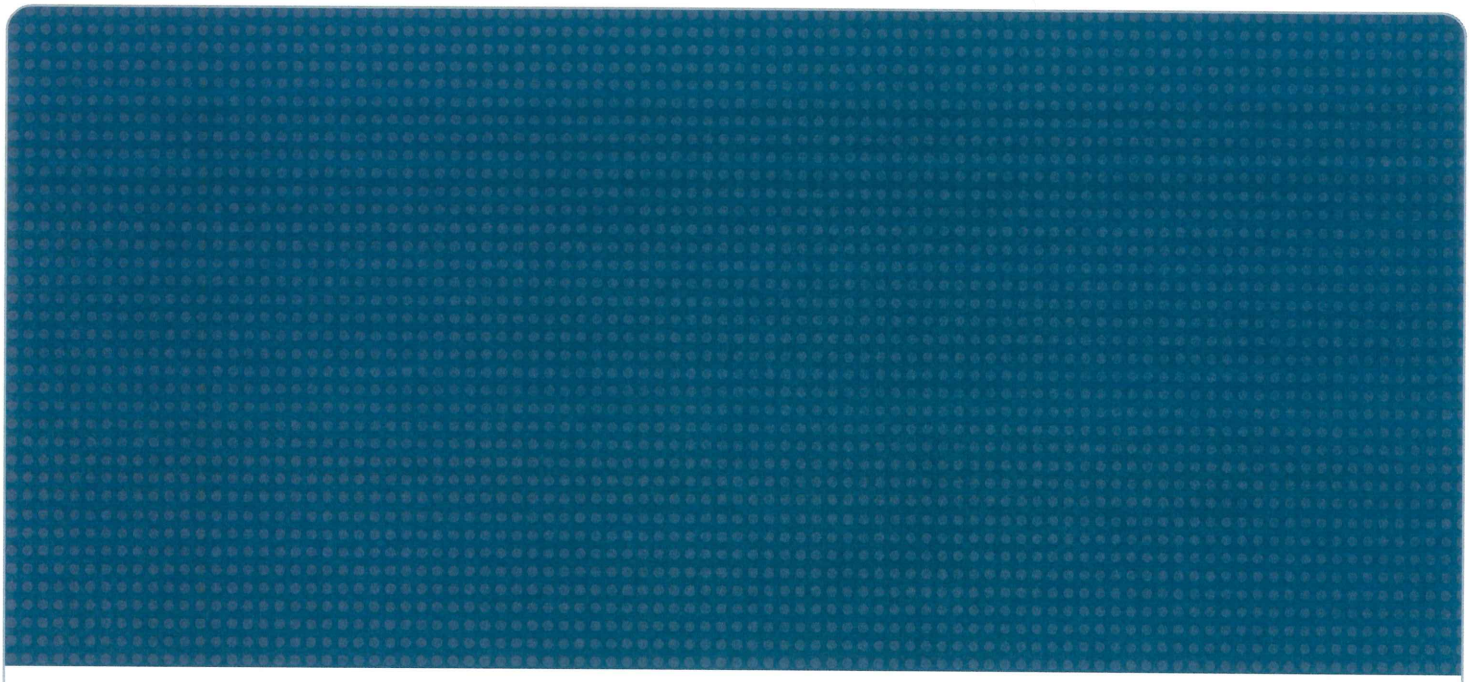
1. Migliaccio AR, et al., Blood 96: 2717-2721, 2000
2. Iori AP, et al., Bone Marrow Transplant 33: 1097-1105, 2004
3. Yoo KH, et al., Bone Marrow Transplant 39: 515-521, 2007
4. Prasad VK, et al., Blood 112:2979-2989, 2008
5. Page KM, et al., Biol Blood Marrow Transplant 17: 1362-1374, 2011

STEMvision™ Instrument # 00087 | Software v 1.3.0.4 | Algorithm 14Day-CordBlood-V 1.2.3 | Stemcell Instruments Registration # 12345



STEMvision™ STEMCELL™ TECHNOLOGIES

**Figure 17: "Parent" Format of the CFU Assay Report Form for CB Cells.**



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## Signature Manifest

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All dates and times are in Eastern Time.

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

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