TECHNICAL MANUAL

STEMvision™

Automated Colony-Forming Unit (CFU) Assay Reader

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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 100V/240V, 50/60 Hz, 1.6A, 1Ph using the AC power cord provided with STEMvision™. Before plugging the instrument in, be sure that a fuse rated 250 V 2A Fast Blow 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™ instrument. In the event of such a spill, disconnect the power cable before cleaning. See section 9.4 for spill cleanup 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. Service 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, 1618 Station Street, Vancouver BC, Canada V6A 1B6. Call 1-604-877-0713 or toll-free 1-800-667-0322 within North America.

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2.0 Introduction

STEMvision™ is an automated instrument and computer system that is designed specifically for imaging, classifying, and counting hematopoietic colonies produced by human or mouse progenitor cells in the colony-forming unit (CFU) assay. For human assays, STEMvision™ 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 [CFU-G], colony-forming units macrophage [CFU-M], and colony-forming units granulocyte-macrophage [CFU-GM]), 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 human STEMvision™ Analysis Packages used for different human CFU assays is described in Table 1. For mouse assays, STEMvision™ can count the total number of colonies in CFU assays (ranging from 7 - 14 days) or colonies derived from erythroid or myeloid progenitor cells in lineage-specific media. A summary of mouse STEMvision™ Analysis Packages is described in Table 2.

STEMvision™ is designed for use with MethoCult™ media (see Table 1 and Table 2 for the appropriate MethoCult™ formulation for different STEMvision™ CFU assays) and meniscus-reducing SmartDish™ cultureware (Catalog #27370). STEMvision™ is optimized for analysis of CFU assays that do not contain background red blood cells (RBCs). RBCs must be cleared from fresh human bone marrow and mobilized peripheral blood samples using HetaSep™ (Catalog #07906) or from human cord blood with HetaSep™ or ErythroClear™ (Catalog #01739) 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 #10000005589) or the Technical Manual: Mouse Colony-Forming Unit (CFU) Assays Using MethoCult™ (Document #10000005597), 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, reproducible results. Each 35 mm culture is imaged in approximately 1 minute and an entire SmartDish™ 6-well plate is imaged in approximately 6 minutes. Analysis can be completed offline in about 6 minutes per plate for human assays or 3 minutes per plate for mouse assays. For high-throughput processing, 20 plates containing 120 cultures can be imaged in about 2 hours, with the image analysis executed overnight. Digital images of the entire culture can be permanently archived for future reference.

For human assays, the results of 7-day and 14-day CFU assays produced by STEMvision™ can be printed 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 for potential future autologous use. Some customization of fields contained within these reports is possible by request.

In the following sections, each of these 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.

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

TISSUE	CFU ASSAY DURATION	COLONY 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)	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)	STEMvision™ Color Human Cord Blood 14-Day CFU Analysis Package (Catalog #22005)	Human CB 14-Day	Lab Parent
		CFU-G/M/GM	MethoCult™ H4035 Optimum without EPO (Catalog #04035)		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)	STEMvision™ Color Human Bone Marrow 14-Day CFU Analysis Package (Catalog #22006)	Human BM 14-Day	Lab
		CFU-G/M/GM	MethoCult™ H4035 Optimum without EPO (Catalog #04035)		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)	STEMvision™ Color Human Mobilized Peripheral Blood 14-Day CFU Analysis Package (Catalog #22007)	Human MPB 14-Day	Lab
		CFU-G/M/GM	MethoCult™ H4035 Optimum without EPO (Catalog #04035)		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.

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Table 2: Summary of STEMvision™ Assays and MethoCult™ Media for Identifying Mouse CFU Types

TISSUE	CFU ASSAY DURATION	METHOCULT™ MEDIUM	COLONY TYPES SUPPORTED BY THE MEDIUM	COLONY TYPES IDENTIFIED BY STEMvision™	STEMvision™ ANALYSIS PACKAGE	STEMvision™ ASSAY TYPE FIELD*
Bone Marrow (BM)	7 - 12 days	MethoCult™ GF M3434 (Catalog #03434)	BFU-E CFU-GM CFU-GEMM	Total CFUs	STEMvision™ Mouse Total CFU Analysis Package** (Catalog #22008)	Mouse Total
	7 - 12 days	MethoCult™ GF M3534 (Catalog #03534)	CFU-GM	Myeloid CFUs	STEMvision™ Mouse Myeloid CFU Analysis Package** (Catalog #22009)	Mouse Myeloid
	7 - 14 days	MethoCult™ SF M3436 (Catalog #03436)	BFU-E	Erythroid CFUs	STEMvision™ Mouse Erythroid CFU Analysis Package (Catalog #22011)	Mouse Erythroid

Abbreviations: BFU-E: burst-forming unit-erythroid; CFU-GM: colony-forming unit-granulocyte-macrophage; CFU-GEMM: colony-forming unit-granulocyte, erythrocyte, macrophage, megakaryocyte; BM: bone marrow.
*Assay types are selected by the user when using the STEMvision™ Analysis application.
**Total CFU and Myeloid CFU algorithms are identical; analysis packages only differ by name and medium used.

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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 (6 x 35 mm wells per plate; Catalog #27370). 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, a heater allows the interior temperature of the acquisition chamber to be 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 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 acquired images, and if desired, manual scoring and editing of automated results

The STEMvision™ software suite is designed for RUO (research use only) applications. However, with the addition of the STEMvision™ 21 CFR Part 11 Compliance Software Add-On (Catalog #500-0110), STEMvision™ can be used in a manner that is consistent with compliance with FDA Title 21 CFR Part 11 regulations on electronic records. For details, refer to section 8.0 of this manual.

STEMvision™ has been optimized for use with SmartDish™ cultureware and MethoCult™ media. It is not recommended for use with different cultureware or media that has not been tested and approved by STEMCELL Technologies. Refer to Table 1 and Table 2 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 at techsupport@stemcell.com.

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3.1 Unpacking and Setup

A STEMCELL Technologies 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™ (section 9.2). Adjustments should only be performed by STEMCELL Technologies qualified personnel.

3.2 Startup

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 indicate 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 minimize 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 (section 4.0).

3.3 Shutdown

1. Turn OFF the heater using the switch located on the front of the instrument (Figure 1B).
2. On the computer, exit the STEMvision™ Acquisition software application.
3. 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.

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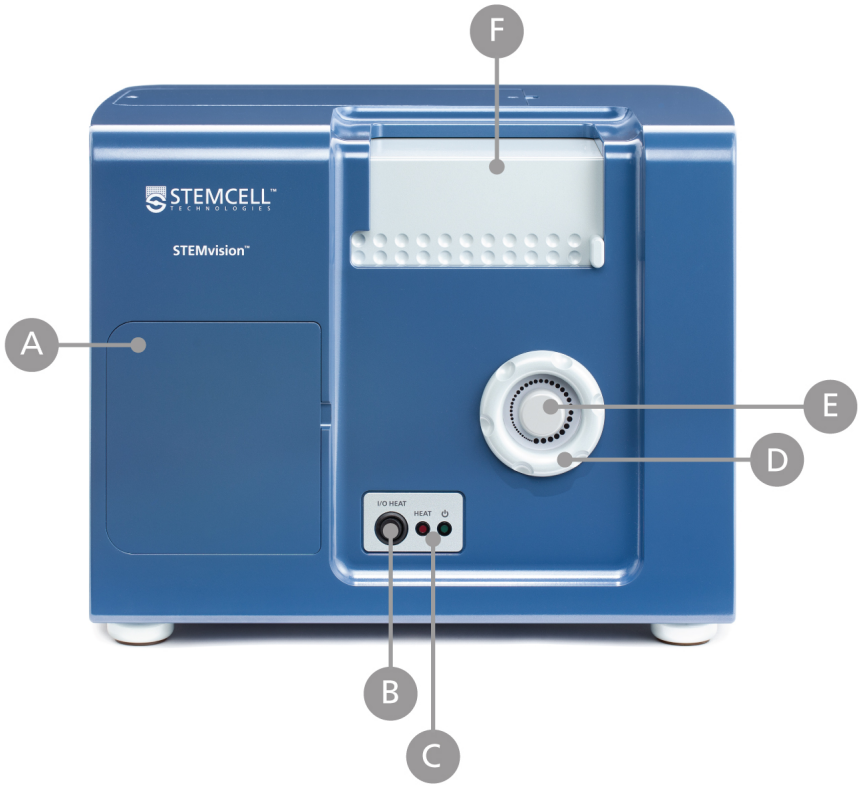

A: Lens access door

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

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A: Power entry module, fuse rating and power switch

B: USB connector

C: Optics door

Figure 2. Back View of the STEMvision™ Instrument

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Figure 3. Front View of the STEMvision™ Instrument with the Enclosure Door Open

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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.

Note: Additional steps may be required for users with the 21 CFR Part 11 Compliance Software Add-On. For more details, refer to section 8.0.

4.1 STEMvision™ Acquisition User Interface Overview

1. Turn ON the STEMvision™ instrument as described in section 3.2.
2. Start the STEMvision™ Acquisition application on the computer by double-clicking the icon on the desktop. The user interface of the STEMvision™ Acquisition application is shown in Figure 4.

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

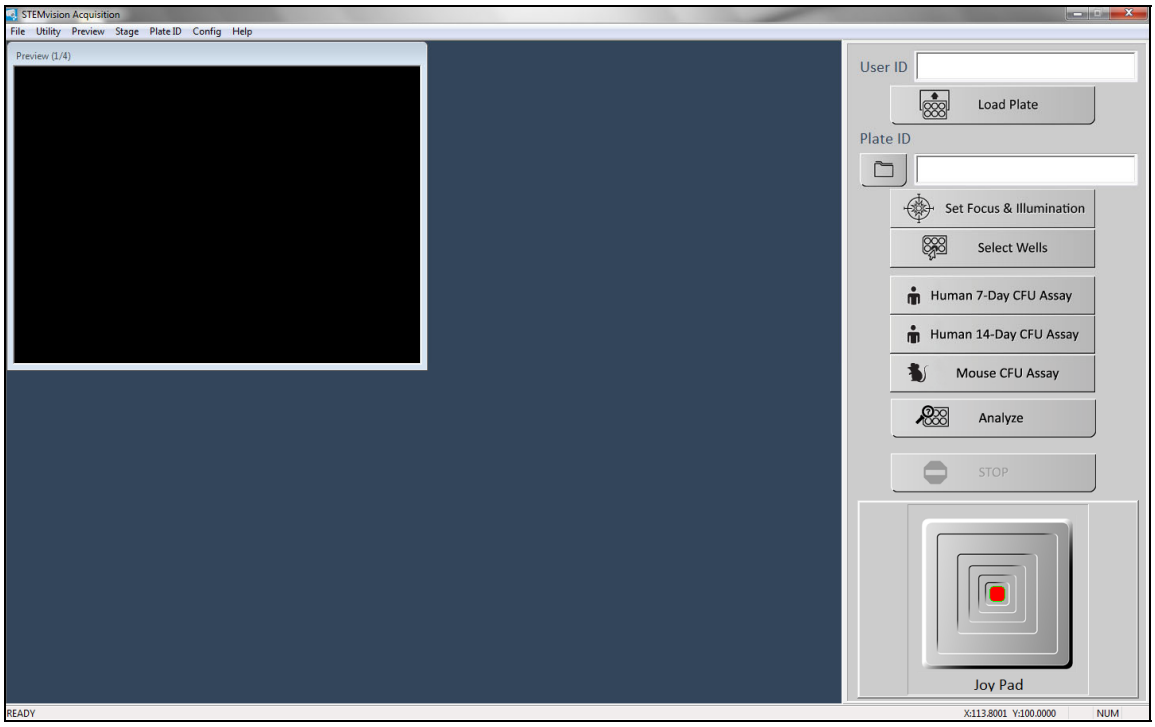

Figure 4. STEMvision™ Acquisition Application on Startup

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 Loading a SmartDish™ Plate

SmartDish™ plates can be imaged with their lids on or off. If plates are to be imaged with the lids on, it is important to ensure that condensation does not collect on the surface of the lids. For more information, see section 4.2.1.

1. Click the "Load Plate" button in the Control Panel to move the stage into the correct position for plate loading.
2. Open the STEMvision™ enclosure door (Figure 1F) and load a SmartDish™ plate onto the sample tray (Figure 3A). 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 affect image acquisition.
3. Close the STEMvision™ enclosure door.

4.2.1 SmartDish™ Incubation Tips

To avoid condensation from collecting on SmartDish™ lids, ensure that the SmartDish™ plates are moved quickly from the incubator to the STEMvision™ instrument. In the event that excessive condensation does appear, users can gently wick away the condensation with a non-abrasive towel.

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.

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- **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 by the Windows User Login name. The User ID is recorded in the acquisition log file (section 7.3).

4.4.2 Sample Identification

Each sample can be identified by Plate ID, Well ID, and Sample ID, as described below. This information is then used in naming acquired images (section 4.4.3), generating log files (section 7.3), and/or the human "Lab" and "Parent" CFU Assay Report forms (section 6.1).

Note: When manually entering Plate ID and Sample ID information, avoid punctuation marks, spaces, and 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-HHMM indicating year, month, day, hour, and minutes, respectively (e.g. 20141103-1331 for November 3, 2014 at 1:31 PM).

Manual Entry - Enter any name in the "Plate ID" field in the Control Panel (maximum 30 characters).

Well ID:

This is generated automatically and corresponds to the wells (A1 through B3) as seen in the Well Navigator dialog box in Figure 5.

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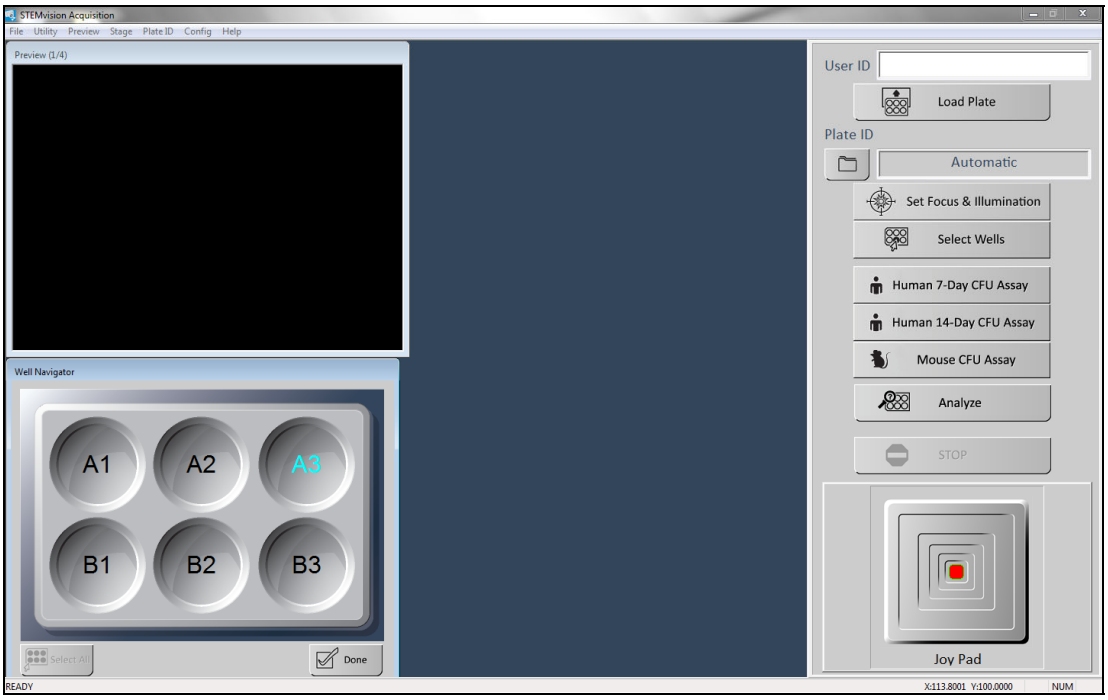

Figure 5. Well Navigator Dialog Box

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.

Important: For human assays, if printed “Lab” or “Parent” CFU Assay Report forms will be required, the sample ID information must be entered via a CSV file.

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Automatic Entry of Sample IDs 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 STEMvision™ computer upon installation of the device software.

1. Ensure a CSV file is populated with data and formatted correctly. The file must consist of 18 comma-separated columns. Refer to Table 3 for guidelines on entering information in the file.

Table 3: Column Properties in the CSV file.

COLUMN	INFORMATION TRACKED IN COLUMNS	FORMAT	EXAMPLE
A	Sample ID	30 characters max	123456ABC
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 Specified) 0 (Female) 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 Paul's
H	Obstetrician Name	30 characters max	Peter Johnson
I	Test Number	30 characters max	T001
J	Number of Nucleated Cells per mL for the sample plated in the CFU assay.	Numeral	12000000
K	Number of Cells Plated per Well	Numeral	20000
L	Pre-processed Volume in µL	Numeral	43000
M	Processed Volume in µL	Numeral	21000
N	Sample Type	-1 = Not Specified 0 = Fresh whole 1 = Fresh processed 2 = Frozen whole 3 = Frozen 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
P	Sample Received Date/time	month/day/year hh:min in numeric format (hours in 24h format)	6/21/2012 17:21:00
Q	MethoCult™ Formulation	30 characters max	H4034
R	MethoCult™ Catalog Number	30 characters max	04034/12E12345

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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 “Add/Modify this Sample ID” at the bottom of the dialog box when finished.
4. Click the “Done” button once the data for all desired wells has been entered.

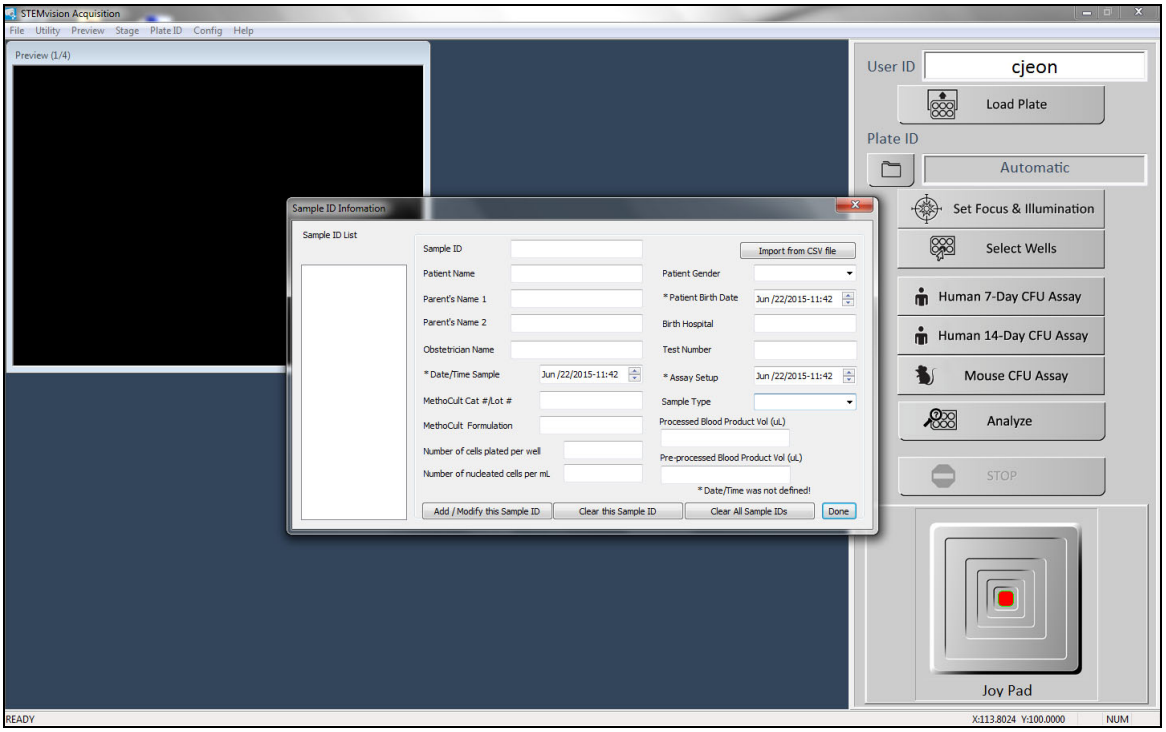

Figure 6. Sample ID Information Dialog Box

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 shutdown of the STEMvision™ Acquisition application. In order to save Sample ID Information, populate a CSV file as described under “Automatic Entry of Sample IDs from a Pre-Populated CSV File”.

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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 customized 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 7.3).

4.4.3 Image Identification

To select the root folder where the acquired images, data log files (section 7.3), and analysis files will be saved, click on the folder icon button in the Control Panel (see Figure 6) 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 filename corresponds to the Plate ID_Well ID.jpg (e.g. Plate1_A1.jpg).

To include the Sample ID in the image filename, click on “Plate ID” in the Menu Bar and select “Include Sample ID in Image File Name”. The image filename will then correspond to 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.

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4.5 Setting Focus and Illumination

The STEMvision™ Acquisition application includes specific tools for properly setting the focus and illumination of the instrument.

Note: When imaging multiple plates, the focus and illumination only needs to be set once before the first plate is imaged. If the instrument has been left unattended, or if the focus knob is moved, the focus should be set again before imaging additional plates.

Note: It is important to ensure that no condensation is present on the lid of the SmartDish™ plate when setting the illumination. If SmartDish™ plates are imaged with the lid off, the exposure time must be reset using a plate with no lid. If the user intends to image the SmartDish™ with the lid off, the focus and illumination procedure must also be performed with the SmartDish™ lid off.

1. Click the “Set Focus & Illumination” button in the Control Panel. Both settings can be adjusted using the same dialog box.
2. If desired, select a specific 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 contains a mostly clear background devoid of any dense colonies (few or no colonies 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 histogram graph falls between the two vertical yellow lines and the “Grey Scale” value reads between 180 and 190. The arrow keys on the PC’s keyboard or the scroll wheel on the mouse can be used for fine exposure adjustments.

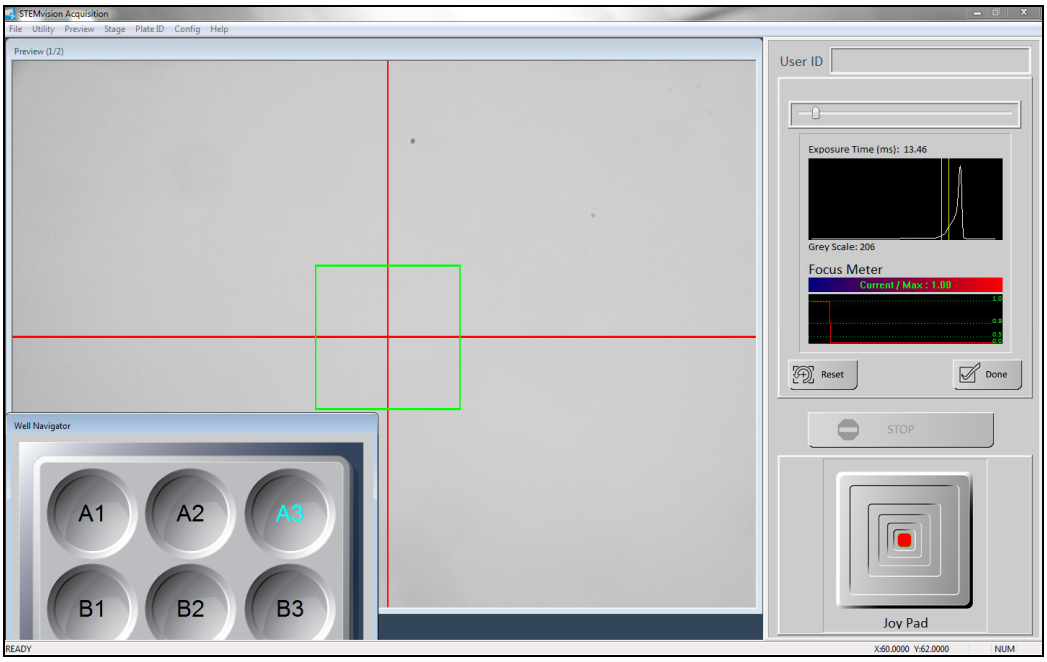

Figure 7. Setting the Illumination in the “Set Focus & Illumination” Dialog Box of the STEMvision™ Acquisition Application

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4. Set the Focus:
    i. Use the Joy Pad to find a location in the well so that a dispersed (e.g. 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. To set the focus for mouse erythroid-specific media, find a location in a well with a dispersed erythroid colony.
    ii. 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 has 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. Use the focus knobs to adjust the focus so that the red line in the live graph approaches a value of 1.0. 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 focus. If necessary, repeat the focus steps with a colony that better represents the average depth of colonies in the MethoCult™ medium.

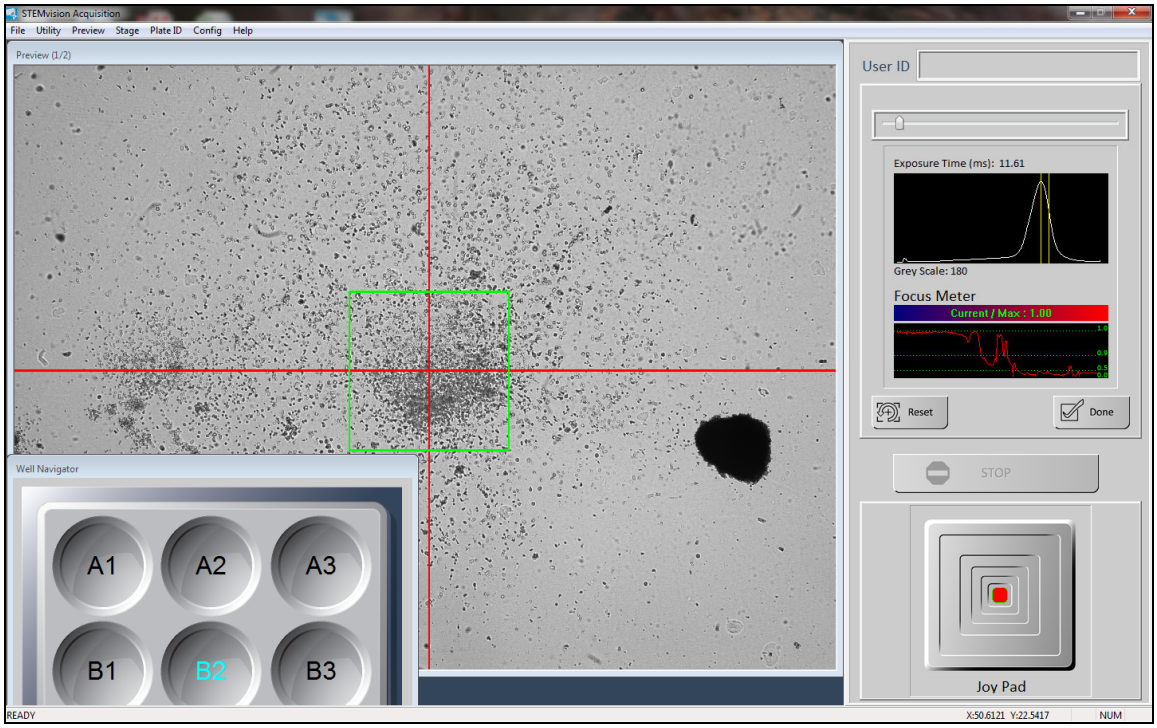

Figure 8. Setting the Focus in the “Set Focus & Illumination” Dialog Box of the STEMvision™ Acquisition Application

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4.6 Image Acquisition

The STEMvision™ Acquisition application produces a composite image of each culture well that comprises 35 separate camera images. The Human 7-Day CFU Assay and all mouse assays will result in a single bright-field image and the Human 14-Day CFU Assay will result in both a bright-field and a dark-field image which can be viewed separately or as a composite color image. All image files are saved to the selected destination root folder (section 4.4.3).

Important: *If printed "Lab" and/or "Parent" CFU Assay Report forms will be required, sample information for each well to be imaged 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. Sample-specific information may be entered for each well if desired.

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 rows 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 dropdown menu or enter information manually. Please note that any information entered in this field will not be saved to a CSV file. Information in this field will be shown in the filename if "Include Sample ID" is selected (see 4.4.3).

   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.

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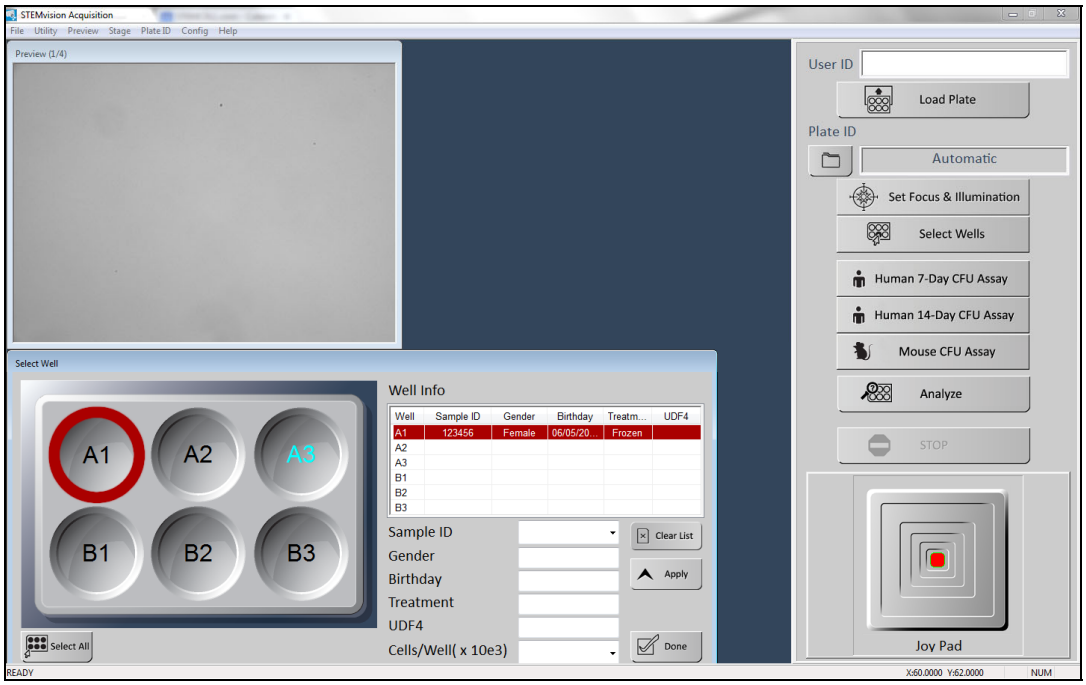

Figure 9. Sample ID Information Dialog Box

4.6.2 Acquiring Images

1. Click the relevant CFU Assay button to acquire images. A real-time preview will show the composite well image being produced as each of the 35 image tiles is sequentially acquired. For “Human 14-Day CFU Assay” acquisition, click on the real-time preview to toggle between the bright-field and the dark-field images being produced.

   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.

   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 4.6.1 and section 4.6.2 step 1.

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

3. To image additional plates, load the next SmartDish™ (section 4.2), select wells (section 4.6.1), and acquire images as described in steps 1 - 2.

   Note: The illumination and focus do not need 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.

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5.0 STEMvision™ Analyzer Application

Note: The Programmed STEMvision™ Dongle must be inserted into one of the computer's USB ports to run the 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 species and tissue types. Therefore, the correct STEMvision™ Analysis Package must be used to analyze images from 7- or 14-day human CFU assays, and from mouse CFU assays. This is because hematopoietic colonies produced by even the same type of progenitor cell from different species or tissues can appear morphologically different. In addition, colonies generated in a 7-day human CFU assay of CB cells performed using MethoCult™ Express (see Table 1) will look different than colonies generated in a 14-day human CFU assay of the same cells performed using MethoCult™ Optimum.

The different STEMvision™ Analysis Packages and the CFU types that they identify are summarized in Table 1 and Table 2. Briefly, the "Color Human BM 14-Day" package identifies 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 "Color Human MPB 14-Day" and "Color Human CB 14-Day" packages identify 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 identified in these tissues. The "Human CB 7-Day" package counts the total number of CFUs in a CB sample without distinction of the different CFU types. Mouse analysis packages count the total number of CFUs according to size, without distinction of colony types. Myeloid or erythroid progenitors can be counted using lineage-specific media.

Note: Analyzing overplated cultures (> 100 colonies/well) or using a 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.

Note: Additional steps may be required for users with the 21 CFR Part 11 Compliance Software Add-On. For more details, refer to section 8.0.

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5.1 User Interface Overview

Start the STEMvision™ Analyzer application on the computer. For human assays, double-click the human icon and for mouse assays double-click the mouse icon on the desktop. The user interface of the human STEMvision™ Analyzer application is shown in Figure 10, and differs only slightly from the mouse STEMvision™ Analyzer application.

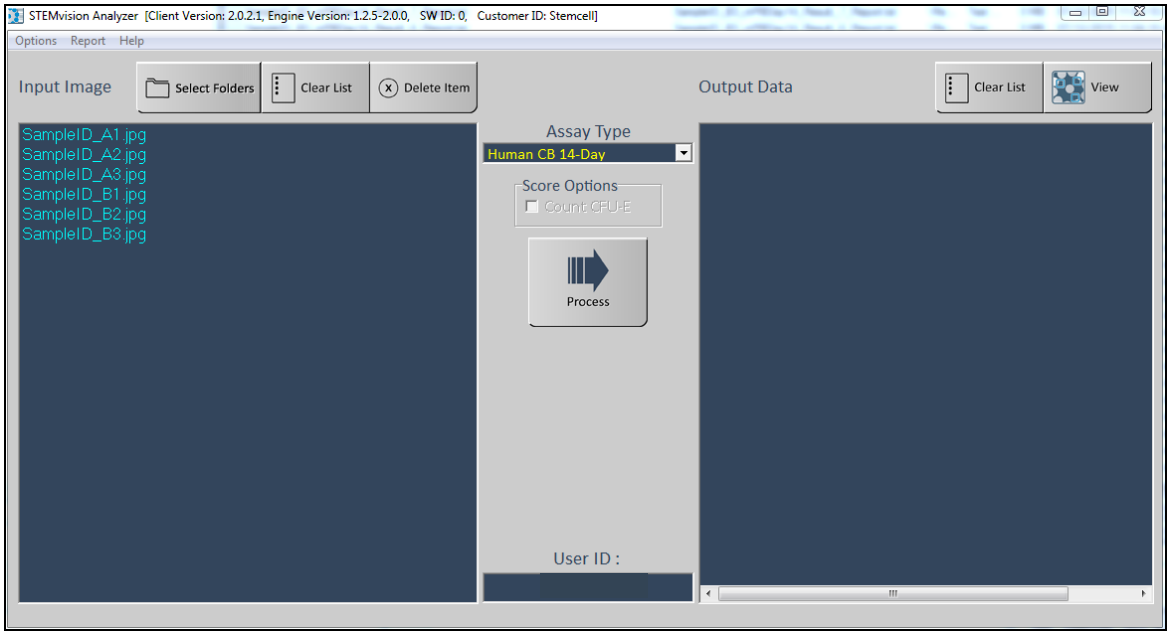

Figure 10. Human STEMvision™ Analyzer Application on Startup

- **Menu Bar:** Located along the top of the application window. It provides access to additional output options such as printing CFU Assay Report forms and data export.
- **Assay Type:** Located in the center of the application window. This is a dropdown 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. The User ID information is recorded in the analyzer report file (section 7.3).

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5.2 Analysis Setup

5.2.1 Generating CFU Assay Report Forms (Human only)

In order to generate “Lab” and “Parent” human CFU Assay Report forms, ensure that a CSV file is populated and imported into the STEMvision™ Acquisition application before acquiring images (section 4.4.2) and that a Sample ID has been defined for each well at the time of acquisition (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 analyses.

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 analyses 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 (Carbon 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 to the Windows default printer, “To PDF” to save an electronic pdf format copy of the report, or “To Printer and PDF”.

   - **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.

   - **Auto Print:** If this option is enabled, the CFU Assay Report forms will be automatically generated and printed to the output format specified after the analysis is complete. When the output format is set to “PDF”, reports for each Sample ID will be saved as a single pdf file. When generating both CFU Assay Report forms (i.e. “Lab” and “Parent”), both reports will be included in the PDF file output. The filename for these reports corresponds to: Sample ID_cells plated per well (x 10^3)_Plate ID_date and time of analysis _Assay Type.pdf. For example:

     Filename: 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.

   - **PDF Output Folder:** Select “PDF Output Folder”, then select the file destination where you want to save the pdf version of the reports.

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5.2.2 Preview of Result Images (Human and Mouse)

Summary result images are automatically generated during image analysis. These images are reduced in size and show outlines of colonies identified by the analysis process. The summary images are saved in the same folder location as the original image and are named with the suffix "_Result_Summary.jpg".

5.3 Image Analysis

5.3.1 Selecting Images for Analysis

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

- **STEMvision™ Analyzer application:** Click on the "Select Folders" button (top left side of the application window) and then browse and select the folder(s) that contains the images to be analyzed (i.e. Plate ID folder). To select multiple Plate ID folders, hold down the SHIFT or CTRL buttons on the keyboard while clicking on the desired folders. All STEMvision™ acquired image files in the selected folder(s) 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. If both a bright field and a dark field image file are present in the folder, only the bright field image will appear in the Input Image field but both will be used in the analysis.
- **Microsoft® Windows® File Manager:** Open a File Manager window and browse to the 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. If the desired analysis is one of the Color Human 14-Day algorithms, ensure that both the bright field and the dark field files are selected before dragging and dropping into the Input Image field.

  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).

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5.3.2 Analyzing STEMvision™ Acquired Images

1. Select the appropriate “Assay Type”. For human assays, select a tissue (BM, MPB or CB), assay duration (7 or 14 days), and assay condition (with or without EPO) from the dropdown menu. To determine which STEMvision™ Analysis Package to use for your application or Assay Type, refer to Table 1 for human assays and Table 2 for mouse assays.

   Note: When using the STEMvision™ Human Bone Marrow 14-Day CFU Analysis Package, counting 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 11).

   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”.

3. As the images are analyzed, the filenames will move from the “Input Image” to the “Output Data” field (Figure 11). 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 filename in the “Output Data” box will be identical to the “Input Image” filename with the addition of “_Assay Type_Result.txt” at the end (e.g. Plate ID_Well ID_Sample ID_hCBDay14_Result.txt). If images are analyzed multiple times, an additional suffix with the analysis number will be added to the result filename.

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 folder (i.e. Plate ID folder). The data files will remain stored in their original location.

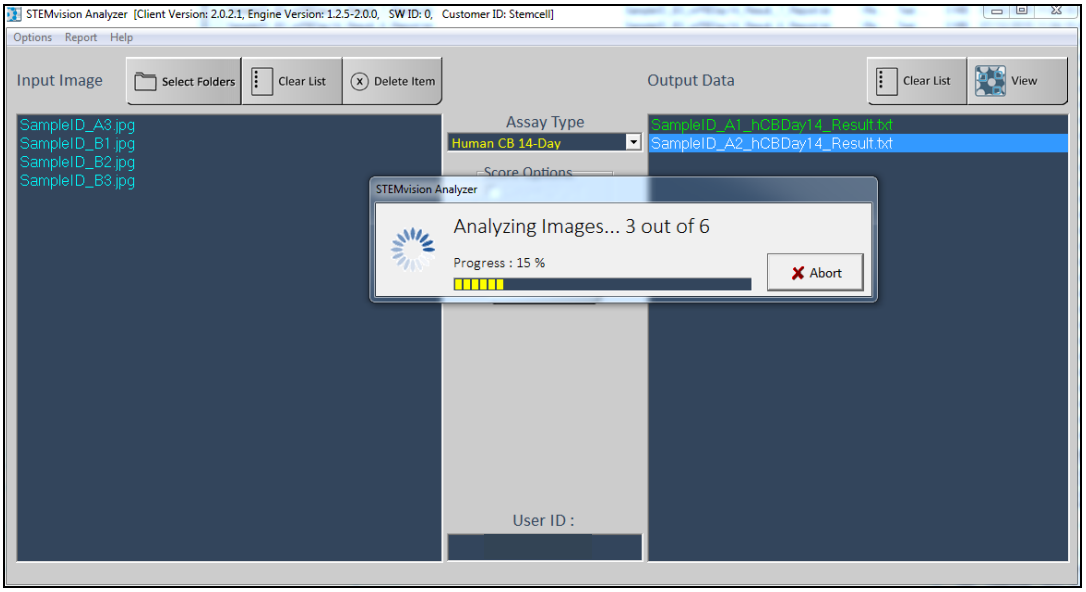

Figure 11. Human STEMvision™ Analyzer Indicating Progress

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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 types of hematopoietic colonies identified by colored circles.
    - For further details on how to view results, see section 6.0.

5.3.3 HL7 Data Export (Human Only)

STEMvision™ Analyzer for human CFU assays has the ability to export colony counts data to a Laboratory Information Management System (LIMS) in accordance with Health Level Seven International Standards (HL7). The exported data includes Sample IDs and the average colony count results from the analysis.

Note: HL7 data export requires that the Auto Print option of the Analyzer application is selected.

To enable HL7 data export, select “HL7 server” from the “Report” menu of the Analyzer application. Enter the IP address and port number of the HL7 server and select “Enable” from the submenu. When enabled, the HL7 message will be automatically transmitted to the server whenever an analysis report for a sample ID is generated. If automatic transmission of the data for each sample is desired, ensure that “Auto Print” is selected from the Report menu. Alternatively, data transmission can be manually initiated for individual samples by generating a printed report using the “STEMvision™ Report Publisher” (see section 6.1).

HL7 data export is not currently available for mouse applications. For further information, please contact your STEMCELL customer service representative.

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6.0 Viewing Results

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

- For human assays, “Lab” and “Parent” CFU Assay Report forms can be generated from samples that have a Sample ID associated with them at the time of image acquisition (section 6.1).
- The STEMvision™ Colony Marker application can be used to view the results of an individual culture image (section 6.2).
- Microsoft® Excel can be used to import a summary table of colony counts (section 7.0).
- The detailed results of the analysis for individual images can be viewed in the analysis result files saved in the image folder. All results files for a given Plate ID can be accessed from the info.html file in the image folder.

Note: Additional steps may be required for users with the 21 CFR Part 11 Compliance Software Add-On. For more details, refer to section 8.0.

6.1 Viewing Human 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
- The “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.

6.1.1 Generating a CFU Assay Report From Previously Analyzed Images

1. In the STEMvision™ Analyzer application, click on “Report” in the Menu Bar and select “Find”. The “STEMvision™ Report Publisher” dialog box will appear (see Figure 12).
2. 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 “Select assay type” dropdown menu. Enter the Sample ID in the search field of the dialog box.

   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.
3. Click the “Find” button. Any Sample IDs that match the query will be shown in the table.
4. Select a desired Sample ID by clicking on the corresponding row in the table.
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 was defined prior to image acquisition.

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6.1.2 Generating a CFU Assay Report From Manually Modified Analysis Results

Automated analysis results may be reviewed and modified in Colony Marker as described in section 6.4. Manually modified results are stored in a cue and may optionally be included in the CFU assay. To include the manually modified results, they must first be imported by the STEMvision™ Analyzer application.

1. In the STEMvision™ Analyzer application, click on “Report” in the Menu Bar and select “Import Results from Colony Marker”. A message box confirming the data import will be shown.

   Note: To permanently clear the queue of manually modified results, click on “Report” in the Menu Bar and select “Reset Colony Marker Import Data”. A message box requesting to confirm the deletion of the queued data is shown. Click “Yes” to clear the current queue of modified data. This will not affect the saved results files or summary images, which will remain in their original locations.

2. Once the modified results have been imported, proceed with generating CFU Assay Reports as described in section 6.1.1. To include the manually modified results, click the box next to “Include results from Colony Marker” in the STEMvision™ Report Publisher dialog box (see Figure 12).

Note: When multiple manually modified results exist for a given image, the most recent result will be used.

Note: Manually modified results are not automatically uploaded to any HL7 server. The updated counts are only sent to an HL7 server (if enabled) when the report is previewed, printed, and/or saved as a PDF.

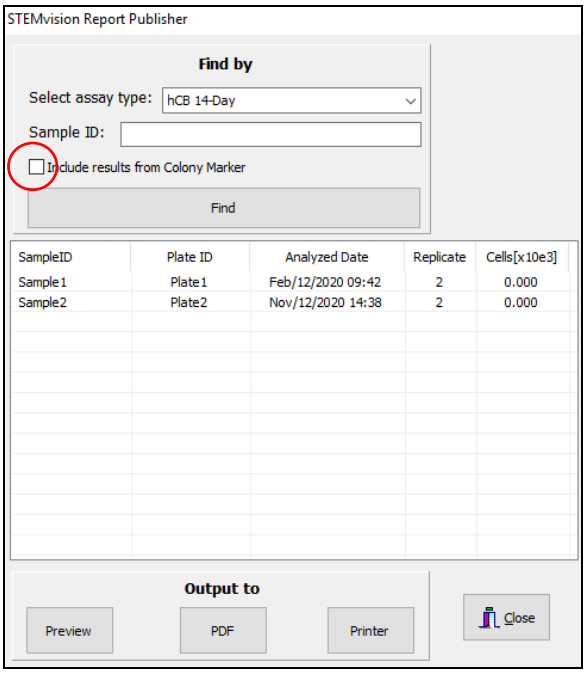

Figure 12. STEMvision™ Report Publisher Dialog Box

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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 (section 5.3.2 step 5).

Note: To edit the results, the image (jpg) and analysis (txt) files must be opened with the STEMvision™ Colony Marker application (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 and does not require the use of the STEMvision™ dongle. If desired, multiple images can be viewed at the same time by having several instances of this application open.

Start the STEMvision™ Colony Marker application on the computer (double-click the icon on the desktop) and choose either human or mouse assay type. The user interface of the STEMvision™ Colony Marker application is shown in Figure 13.

Figure 13. Human STEMvision™ Colony Marker Application on Startup

- **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. For human assays it shows

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the summary of CFU type (i.e. CFU-E, BFU-E, CFU-G/M/GM or CFU-GEMM), and for mouse assays the summary of CFU size class (i.e. size class 1, 2, etc.) that has been scored by STEMvision™.

- **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.

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). Human 14-Day dark-field images may be opened separately, if desired. If the selected file is a bright-field image and there is a corresponding dark-field image in the folder, the image shown in the preview window will be a color composite of both the bright-field and dark-field images.

   Note: Images may be dragged and dropped directly from a Windows® 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 Windows® File Manager window into the “Colony List” field.

   Colonies will be marked on the image as shown in Figure 14 (human) or Figure 15 (mouse).

Note: A scale bar may be displayed on the image by clicking on “Image” in the Menu Bar and selecting “Scale Bar”. The scale bar is displayed in the lower right corner of the View window, and shows the zoom level and size (mm) that the scale bar represents.

For human assays, the colored circles around each colony correspond to the CFU type that has been scored by STEMvision™, as outlined below:

Human assays (Figure 14)

Red: BFU-E

Yellow: CFU-G/M/GM

Blue: CFU-GEMM

Orange: CFU-E

Green: No colony type (i.e. used in STEMvision™ Human Cord Blood 7-Day CFU Analysis Package to count total CFUs)

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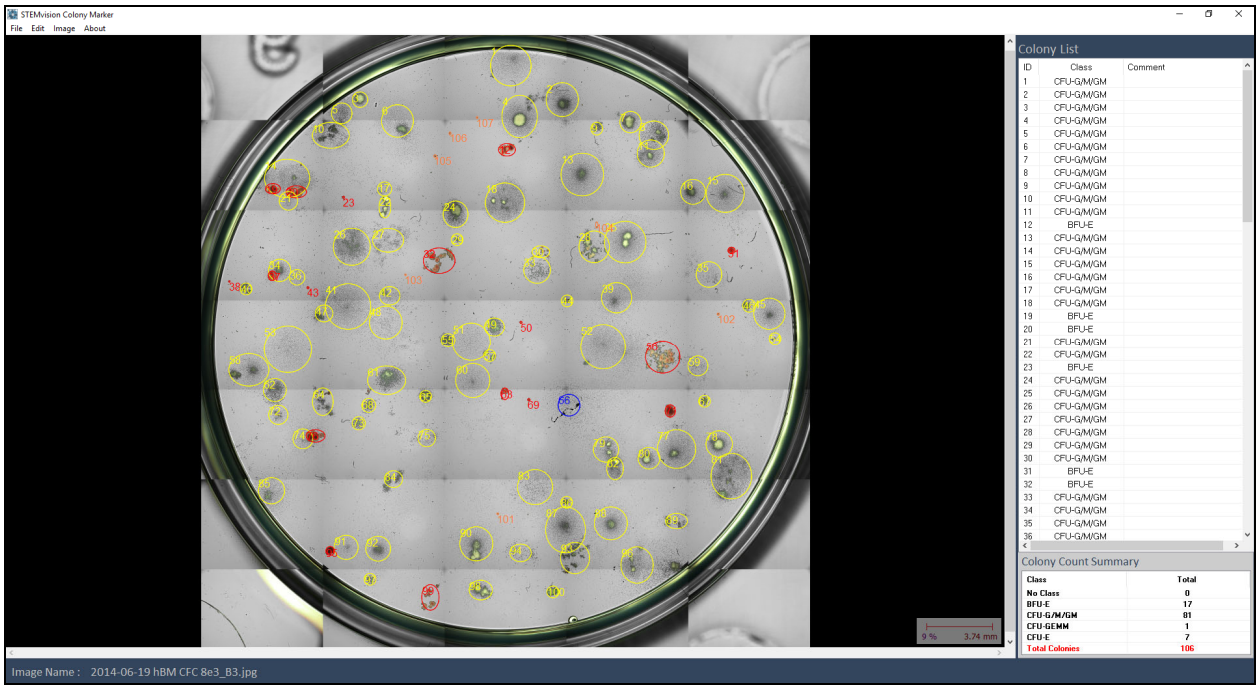

Figure 14. Image and Analysis of a Human CFU Assay Using the STEMvision™ Colony Marker Application

This image is from a 14-day CFU assay on human BM cells that was analyzed with the STEMvision™ Color Human Bone Marrow 14-Day CFU Analysis Package. Red circles identify BFU-E-derived colonies; yellow circles identify CFU-G/M/GM-derived colonies; blue circles identify CFU-GEMM-derived colonies; and orange circles identify CFU-E-derived colonies.

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For mouse assays, the colored circles around each colony correspond to the CFU size class that has been scored by STEMvision™. Four size classes (class 1 [smallest] to class 4 [largest]) are based on colony size and density (outlined below). This feature allows researchers to define size cutoffs so that very small colonies can be excluded from total colony counts if desired.

Mouse assays (Figure 15)

Red: Size class 1, smallest colonies

Yellow: Size class 2

Blue: Size class 3

Orange: Size class 4, largest colonies

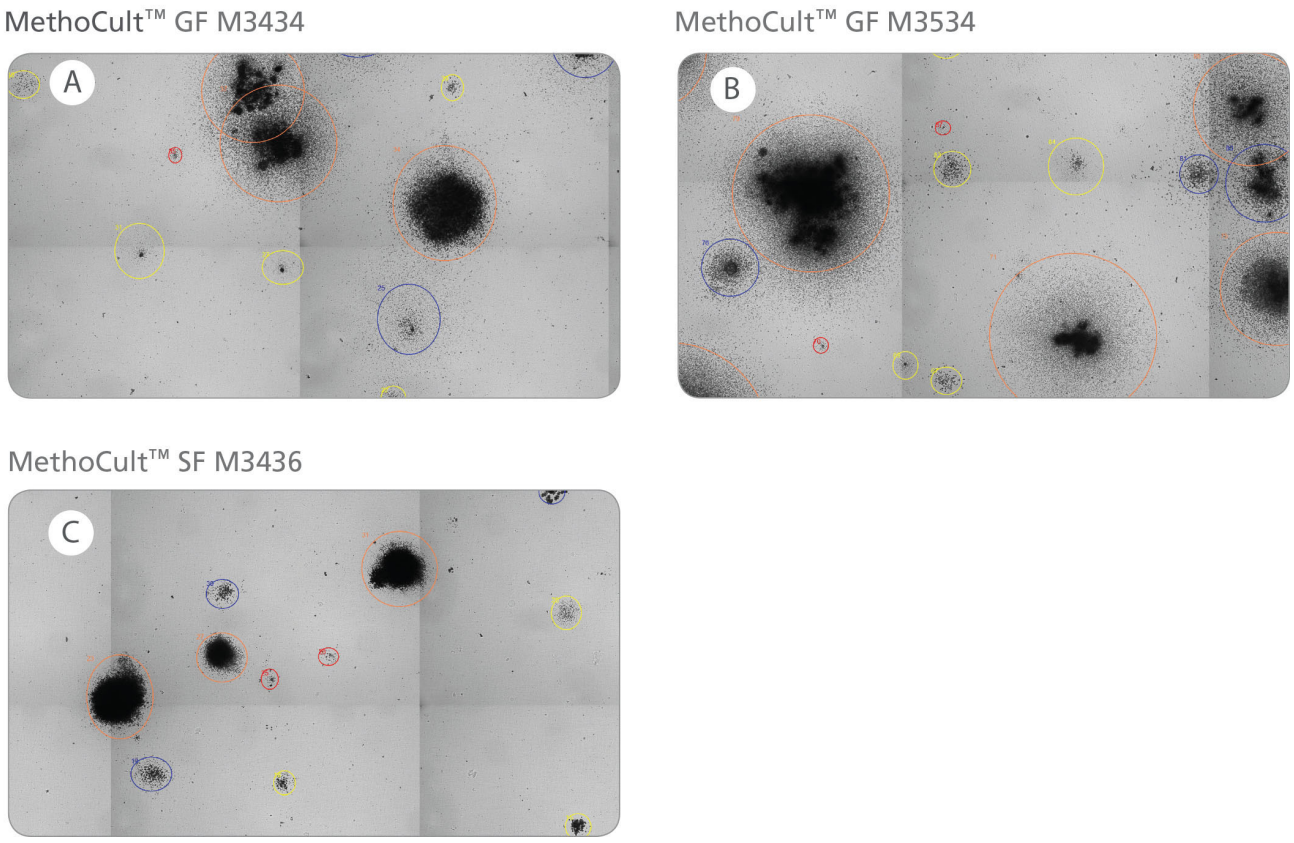

Figure 15. Images and Analysis of Mouse CFU Assays in Three Types of MethoCult™ Media
Images were acquired using STEMvision™ after 12 days of culture of mouse BM cells in **(A)** MethoCult™ GF M3434, **(B)** MethoCult™ GF M3534, and **(C)** MethoCult™ SF M3436, and analyzed using the corresponding Mouse Analysis Package for each medium. Red circles identify the smallest colonies (size class 1), yellow circles identify size class 2, blue circles identify size class 3, and orange circles identify the largest colonies (size class 4).

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Additional Colony Marker functions are accessible via the right-click context menu displayed by right-clicking on the image (Figure 16), or by designated “Hot Keys” described in section 6.3 step 3iii.

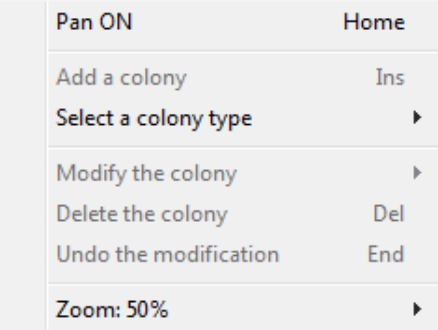

Figure 16. STEMvision™ Colony Marker Context Menu

At any time, the zoom level of the image can be adjusted by selecting a zoom level from the context menu, or by rolling the mouse wheel button up or down. To navigate through the image, select “Pan ON” from the right-click context menu (Figure 16), or press the “Home” key. The cursor will change to a hand icon which can then be used to grab and drag the image by pressing the left mouse button while moving over the image. To return to the “select” mode, press the “Home” key once again or select “Pan OFF” from the context menu.

6.3 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. To add a new colony:
   i. Right-click the mouse and select “Add a colony” or press the “Insert” key. A brown circle, with its ID number, will appear where the cursor is located.
   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 type that corresponds to the new colony by right-clicking and selecting it from the options listed under the “Select a colony type” menu, or use the appropriate keyboard shortcut:

Human assays	Mouse assays
F1: BFU-E	F1: Size class 1
F2: CFU-G/M/GM	F2: Size class 2
F3: CFU-GEMM	F3: Size class 3
F4: CFU-E	F4: Size class 4
F5: No colony type	F5: No size class

   Note: To cancel addition of a new colony, select the colony by clicking on it, then press the Delete key.
   iv. Repeat steps i - iii until all of the additional colonies have been added.

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Note: The Colony Count Summary field will be automatically updated with every change made to the results.

4. Save the results by clicking on “File” in the Menu Bar and select “Save Data.” A prompt for a filename and destination folder will pop up. The data are saved in the form of a .txt file.

   Note: When entering filenames, avoid punctuation marks, spaces, and characters not compatible with the Microsoft® Windows® file naming system, such as commas, “/”, “\”, “”, and “?”.*

   Note: If manually scored results are to be included in summary tables compiled in Microsoft® Excel, the results filename must include the word “Manual”.

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: Manual edits to STEMvision™ analysis results are temporarily stored in a queue and can be imported by the Analyzer application. To include manually modified results in the “Lab” or “Parent” CFU Assay Report forms, follow the steps outlined in section 6.1.2.

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, follow the steps outlined in section 6.3 step 3.

   Note: The Colony Count Summary field will be automatically updated with every change made to the results.
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. Complete the change by re-specifying the colony classification.

   Note: To cancel the edits, select “Undo the modification” from the right-click context menu or press the “End” key.
4. To change a colony’s CFU type:
   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 type by selecting a colony type from the “Modify the colony” option in the right-click context menu or pressing one of the function hot keys (see section 6.3 step 3.iii). The outline color will change to the appropriate colony color indicating that a change has been made.

   Note: To cancel the edits made to the colony sub-type, right-click and select “Undo the modification” or press the “End” key.
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. Select “Delete the colony” from the right-click menu or press the “Delete” key.

Note: Save the updated data file as a separate file from the original that is produced automatically by the STEMvision™ Analysis application. To do so, click on “File” in the Menu Bar and select “Save Data.” A prompt for a filename and destination will appear. By default, the new filename will be the original filename with “_Mod” added to the end.

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7.0 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. Summary tables, as shown in Figure 17 and Figure 18, will be generated.

Image Name	Total E	Total M	Total	CFU-GEMM	CFU-GM	BFU-E	CFU-E	Application	Errors
Plate2_B2_mPBDay14_Result.txt	7	12	19	1	11	7	0	MPB	Colony number below target range;
Plate2_A2_mPBDay14_Result.txt	20	0	20	0	0	20	0	MPB	
Plate1_B2_mPBDay14_Result.txt	10	7	17	1	6	10	0	MPB	Colony number below target range;
Plate1_A3_mPBDay14_Result.txt	10	19	29	2	17	10	0	MPB	
Plate1_A2_mPBDay14_Result.txt	2	12	14	0	12	2	0	MPB	Colony number below target range;
Plate1_A1_mPBDay14_Result.txt	8	8	16	1	7	8	0	MPB	Colony number below target range;
Plate2_B2_hBMDay14_Result.txt	7	11	18	1	10	7	0	hBM	Colony number below target range;
Plate2_A2_hBMDay14_Result.txt	18	2	20	0	2	18	0	hBM	
Plate1_B2_hBMDay14_Result.txt	10	7	17	1	6	10	0	hBM	Colony number below target range;
Plate1_A3_hBMDay14_Result.txt	10	22	32	2	20	10	0	hBM	
Plate1_A2_hBMDay14_Result.txt	2	12	14	0	12	2	0	hBM	Colony number below target range;
Plate1_A1_hBMDay14_Result.txt	8	8	16	1	7	8	0	hBM	Colony number below target range;
Plate2_B2_hCBDay14_Result.txt	6	11	17	1	10	6	0	hCB	Colony number below target range;
Plate2_A2_hCBDay14_Result.txt	18	0	18	0	0	18	0	hCB	Colony number below target range;
Plate1_B2_hCBDay14_Result.txt	8	7	15	1	6	8	0	hCB	Colony number below target range;

Figure 17. Example of a Summary Table for the Human Analysis Package Generated in Microsoft® Excel

Image Name	Class 1	Class 2	Class 3	Class 4	Total	Application	Errors
2015-06-08 M3434-1_A1_05_mTotal_Result.txt	5	5	5	1	16	mTotal	
2015-06-08 M3434-1_A2_05_mTotal_Result.txt	2	5	10	2	19	mTotal	
2015-06-08 M3436-1_A1_2_mErythroid_Result.txt	10	10	6	0	26	mErythroid	
2015-06-08 M3436-1_A2_2_mErythroid_Result.txt	4	2	7	3	16	mErythroid	
2015-06-08 M3534-2c_B2_3_mMyeloid_Result.txt	11	24	32	11	78	mMyeloid	
2015-06-08 M3534-2c_B3_3_mMyeloid_Result.txt	10	26	28	12	76	mMyeloid	

Figure 18. Example of a Summary Table for the Mouse Analysis Package Generated in Microsoft® Excel

The name(s) of the analyzed image(s) is (are) shown in the first column, and the counts for each colony type are shown in the subsequent columns.

For human image analysis, “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). For mouse image analysis, colonies are classified by size into one of four classes, with “Class 1” referring to the smallest. The column labeled “Total” gives 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 setup 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 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

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uneven dispersion or dehydration of MethoCult™ medium). Multiple error messages will be separated by a semicolon.

CFU assay summary tables can be created for individual plates, individually selected wells, or based on specific search criteria, as described below.

Note: Any existing content in the Microsoft® Excel spreadsheet that overlaps with the resulting table will be deleted. For images analyzed with the STEMvision™ Human Cord Blood 7-Day CFU Analysis Package that does not score the CFU types or size classes, values will be shown in the "Total" colony counts column only.

7.1 Creating a Summary Table for Individual Plates or Wells

1. From within a Microsoft® Excel spreadsheet, right-click on the cell where the summary table is to be inserted and select "STEMvision™: Select results folder" from the dropdown menu.
2. Browse to and select the image folder (i.e. Plate ID folder) that contains the analysis result files.
3. 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).
4. Click the "Open" button to compile the summary table for the selected results.

7.2 Creating a Summary Table Based on Search Criteria

1. From within a Microsoft® Excel spreadsheet, right-click on the cell where the summary table is to be inserted and select "STEMvision™: Select results folder" from the dropdown menu.
2. Browse to and select the image folder (i.e. Plate ID folder) that contains the analysis result files.
3. In the search box at the top right of the file dialog, type the search criteria of interest (use '*' for wild card searches). All results files that match the search criteria will be displayed in the dialog box.

   *For example, to list all the results for images analyzed with the STEMvision™ Color Human Cord Blood 14-Day CFU Analysis Package, you may enter "*hCBDay14*" into the search field.*
4. 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.
5. Click the "Open" button to compile the summary table for the selected results.

7.3 Acquisition and Analyzer Logs

Information for each image acquisition and analysis session is logged by STEMvision™ in an automatically generated "Info" file. The "Info" file contains:

- Acquisition logs, with the following information related to image 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, with the following information related to image 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 folder (i.e. Plate ID folder) in the root folder. Within this folder, locate the file named "Plate ID_Info.htm" ("Plate ID" is the name entered for the plate during image acquisition). Open the file to display the acquisition and analysis information for the plate.

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8.0 Use of STEMvision™ in a 21 CFR Part 11-Compliant Manner

8.1 Introduction

In 1997, the United States Food and Drug Administration (FDA) introduced a set of regulations that stipulate rules and guidelines for ensuring the security, integrity, and authenticity of electronic data generated by devices such as instruments used in regulated environments, FDA Title 21 CFR Part 11 (21 CFR Part 11).

The STEMvision™ software suite is designed for RUO (research use only) applications. With the addition of the STEMvision™ 21 CFR Part 11 Compliance Software Add-On (Catalog #500-0110), STEMvision™ can be used in a manner that supports compliance with 21 CFR Part 11 regulations on electronic records. This is implemented via a combination of system access security policies, protection of data from alteration/falsification/deletion, recording of system- and application-level events in protected log files, ability to generate audit trails, and electronic signing of data reports.

This section describes setup, use, and limitations of STEMvision™ for supporting users in meeting the requirements of 21 CFR Part 11 in Windows 7 and Windows 10 environments.

8.2 Definitions Related to 21 CFR Part 11-Enabled Software

TERM	DEFINITION
STEMvision™ System	The STEMvision™ instrument, STEMvision™ computer, and STEMvision™ software together comprise the STEMvision™ system
Standard Settings	In this software state, security policies according to the controls specified in 21 CFR Part 11 are NOT implemented and/or enforced
21 CFR Part 11-Enabled	In this software state, security policies according to the controls specified in 21 CFR Part 11 are implemented and enforced
Electronic Data Record	An entry in the STEMvision™ Database
Event record	A STEMvision™ application event recorded in STEMvision™ Application Log files and Windows Event Log (WEL)
Result File	A CSV delimited text file that is a tabular listing of the colonies identified by the STEMvision™ Analyzer
Info File	A text file generated by STEMvision™ Acquisition containing the properties and sample information of an imaged plate
Checksum	A sum derived from the bits of data in a STEMvision™ data file that is calculated before and after transmission or storage to assure that the data are free from errors or tampering. STEMvision™ employs the MD5 hash function algorithm to calculate checksums. Appears as a long string of numbers and letters in the Audit Trail Viewer.

TERM	DEFINITION
CFU Assay Report (“Lab” or “Parent”; see section 11.0)	The final report produced by the report generator of STEMvision™ Analyzer; contains the sample information, summary data, summary images
Log File	A file that records the events generated by each of the STEMvision™ applications. Using standard settings, log files are in the format of CSV files. With 21 CFR Part 11-enabled software, log files are encrypted as binary files. Each application generates a new log file when the application is launched.
Audit Trail	The collection of STEMvision™ application events (Table 5) that are tracked as log files. Logged events can be parsed as an audit trail using the STEMvision™ Audit Trail Viewer (see section 8.7), and output to a report.
Event Viewer	The Windows Event Viewer. Allows review of all logged STEMvision™ application events from the Windows Event Log (WEL). The Windows Event Log captures the complete set of STEMvision™ events and is accessible to all users.
Audit Trail Viewer	A STEMvision™ application log viewer that allows users to generate an audit trail directly from the encrypted STEMvision application log files
Electronic Signature	An electronic verification of a CFU Assay Report, by a user entering their unique credentials (identification and password) at the time of signature placement. The signature (i.e. verification of user credentials) is stored with a timestamp in the event log linked to the report being verified. This function is only available in 21 CFR Part 11-enabled software.
Local User	A user that is logged into the STEMvision™ system in the local domain of the STEMvision™ computer
Network User	A user that is logged into the STEMvision™ system in a network environment based on Windows Active Directory domain services
User Group	A collection of multiple user accounts that share the same access rights to the STEMvision™ system and have common security rights. The default STEMvision™ user groups are “STEMvision™ Users” and “STEMvision™ Admins”. Access restrictions for each of these groups are summarized in Table 4 (section 8.3.1).
Closed System	The STEMvision™ system when not connected to a network; all system, application, and data files are stored on the local drive of the STEMvision™ computer
Open System	The STEMvision™ system when connected to a network, where the network provides and verifies the user credentials. System and application files reside on the local drive of the STEMvision™ computer, while data may reside either on the local drive or networked locations.

8.3 Setup and Installation

Once purchased, the STEMvision™ 21 CFR Part 11 Compliance Software Add-On will be installed by your STEMCELL service representative; no further software installation is required by the user.

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8.3.1 User Groups

User group permissions and security policy settings have been optimized to support compliance to 21 CFR Part 11 regulations. Changing these permissions and security policies may invalidate the security features of the software and result in a non-compliant workflow. Contact your STEMCELL sales representative for further information.

Three user groups have been defined, two of which are intended to support a 21 CFR Part 11-compliant workflow: **STEMvision™ Users** and **STEMvision™ Admins**. Access restrictions have been specifically defined for these user groups with the intent of limiting the ability of STEMvision™ Users to accidentally or willfully edit data and reports. See Table 4 for details on permissions for each of the user groups.

The third user group is **System Administrator** (Windows Administrator). System Administrators have complete and unrestricted access to the STEMvision™ system, and cannot be users of STEMvision™ in a 21 CFR Part 11-compliant workflow. Generally, only Information Services (IS) personnel—with no stake in the use of the instrument for generating data—are System Administrators. The System Administrator may add and remove users from the STEMvision™ user groups.

Table 4. Access Restrictions for Default User Groups

	STEMvision™ USERS	STEMvision™ ADMINS	SYSTEM ADMINISTRATOR
Acquisition	Full access		
Analyzer	Report options are limited to report type (Lab and/or Parent)	Full access to all menus	
Colony Marker	Full access		
File System	• Full permissions to C:\CFC root folder • Restricted access to C:\CFC\History and subfolders • Restricted access for Windows\System folders	• Full permissions to C:\CFC root folder and subfolders • Restricted access for Windows\System folders (confirm)	Full access
System Processes	• 'View only' access to Windows Event Log/ STEMvision™ Event Viewer • No access to STEMvision™ Application Log via the Audit Trail Viewer • No access to STEMvision™ database • 'View only' access to STI registry keys • Restricted from adding/editing/removing users or groups • Restricted from editing security policy	• 'View only' access to Windows Event Log/STEMvision™ Event Viewer • 'View only' access to STEMvision™ Application Log via the Audit Trail Viewer • No access to STEMvision™ database • 'View only' access to STI registry keys • Restricted from adding/editing/removing users or groups • Restricted from editing security policy	• Full access to Windows Event Log/STEMvision™ Event Viewer • 'View only' access to STEMvision™ Application Log via the Audit Trail Viewer • No access to STEMvision™ database • Full access to STI registry keys • Full access for adding/editing/removing users or groups • Full access to security policy

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8.3.2 Adding Local Users

Note: Only System Administrators can add local users.

1. Open the Windows “Local Users and Groups” editor (in the search bar, enter lusrmgr.msc and press ENTER).
2. Right-click on the “Users” folder icon in the “Local Users and Groups” list on the left side of the local users editor window and select “Add User”.
3. Enter a User ID and, if desired a full name/description. A password may be assigned, or the user may set their own password on first login.
4. Click the create button to add the user to the users list.
5. Additional users may be added in the same manner. Click the close button when all users have been added.

8.3.3 Adding Users to User Groups

Note: Only System Administrators can add users to user groups.

1. Click on the “Groups” folder icon in the “Local Users and Groups” list on the left side of the local users and groups editor window.
2. In the middle panel of the editor window, double-click on the user group to be edited. A Users Properties dialog box will appear.
3. Click the “Add…” button and enter the user’s name to be added to the group in the object names box. Multiple users may be entered into the object names box, separated by a semicolon. Click OK to add the specified users to the group.

8.3.4 Removing Users from User Groups

Note: Only System Administrators can remove users from user groups.

1. Click on the “Groups” folder icon in the Local Users and Groups list on the left side of the “Local Users and Groups” editor window.
2. In the middle panel of the editor window, double-click on the user group to be edited. A Users Properties dialog box will appear.
3. Select the user to be removed from the list of members in the Users Properties dialog box. Multiple users may be selected by holding the CTRL key while selecting.
4. Click the “Remove” button to remove the selected users from the group.

8.3.5 Setting Password Policy

Note: Only System Administrators can set the password policy.

1. Open Windows Group Policy editor (in the search bar, enter gpedit.msc and press ENTER).
2. On the left panel of the editor, navigate to Computer Configuration → Windows Settings → Security Settings → Account Policies.
3. Click on ‘Password Policy’ in the left panel. Policies for password length, age, and complexity may be set as desired.

   Note: Additional security policies may be defined. Consult with your organization’s IS department or contact your STEMCELL representative for more information.

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8.3.6 Use with Active Directory

The STEMvision™ system may be bound to a network domain using Windows Active Directory. The system has been validated for use with Active Directory to meet all the system access controls defined in 21 CFR Part 11. Consult with your organization's IS department or contact your STEMCELL representative for more information.

8.4 Logging In and User Authentication

To use the STEMvision™ system, a user must log in using standard Windows procedures. Enter your user ID and password on the Windows login screen. Once logged in, STEMvision™ applications may be used as described in this manual. Features and access will be limited depending on the user group to which the currently signed-in user belongs.

Note: To operate the STEMvision™ instrument and software, the user must belong to either STEMvision™ Users or STEMvision™ Admins. Permissions for each user group are summarized in Table 4 (section 8.3.1).

8.5 Acquiring and Analyzing Images

The standard procedures for acquiring and analyzing images described in sections 4.0 and 5.0 also apply when the instrument is operated with 21 CFR Part 11 software enabled. However, there are additional restrictions imposed on the STEMvision™ Users group. These are summarized in sections 8.5.1 and 8.5.2.

8.5.1 Acquisition

The default folder for storing raw data (images and analysis results) is set to C:\STEMvisionData. It is recommended to save all data to this folder. However, members of both STEMvision™ Users and STEMvision™ Admins may override the default directory. A warning message that changing the default data path is not compliant with a 21 CFR Part 11 workflow is displayed the first time a new data folder is chosen.

8.5.2 Analyzer

STEMvision™ Users are not permitted to change report format (types, header information, and output format). In addition, STEMvision™ Users cannot change the database or enable/configure export to HL7 server.

8.6 Report Generation

The report generation procedure is described in section 6.1.

21 CFR Part 11-enabled STEMvision™ software requires reports to be signed by the currently authorized user and/or a STEMvision™ Admins user. Reports may be previewed without being authenticated by a user signature, but cannot be saved or printed until at least one signature is registered.

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8.6.1 Report Setup

STEMvision™ Admins

Only a STEMvision™ Admins user may specify the report format. The report header information (Institute Information), receiver title, and CC name can be specified as described in section 5.2.1. Additional formatting options (output format, paper size, and report type) and output folder may also be edited by STEMvision™ Admins users. Any changes made by the STEMvision™ Admins user will apply to future reports generated by STEMvision™ Users.

STEMvision™ User

The STEMvision™ User is not permitted to edit report format options.

8.6.2 Report Generation

STEMvision™ Users or STEMvision™ Admins

To generate a report, open the Analyzer application and click on the Report menu in the application menu bar. From the menu, choose Find. Identify and select the sample ID for which a report is desired (see section 6.1.1). Click either the PDF or Printer button to send the report to a PDF file or printer. A report approval dialog will appear, with Technician approval and Lab Manager approval sections. The currently logged-in user ID is displayed in the Technician ID field. To sign the report, the user must enter their password into the Password field and click the Approve button.

A Lab Manager (i.e. member of STEMvision™ Admins) may additionally sign the report by entering their credentials into the ID and Password fields, and clicking the Approve button. The manager need not be logged in to approve the report, and may add their approval while a STEMvision™ User is signed into the system.

Once a signature has been authenticated, the “Proceed to Print” button is enabled. Click this button to generate the report and send it to the specified output format. Only one signature is required (either Technician or Lab Manager) to print the report. The user ID and time stamp of the report authentication is shown in the signature lines of the resulting report.

While the signatures are tracked in the STEMvision™ audit trail, a new signature is required each time a report is generated.

8.7 Audit Trails

21 CFR Part 11-enabled STEMvision™ software generates a secure log of STEMvision™ application events—the **STEMvision™ Application Log** (section 8.7.1). STEMvision™ Application Logs are encrypted and can only be accessed using the Audit Trail Viewer. In addition, the **Windows Event Log (WEL)** generates a secure log of operating system events (section 8.7.2). WELs can be viewed using the Audit Trail Viewer application. All STEMvision™ application events are recorded in the WEL.

8.7.1 STEMvision™ Application Log

Each of the STEMvision™ software applications log key application level-user activities, termed “events”.

Each event has, at minimum, an associated timestamp, event type, application that generated the event, and the current user. A detailed description of each event type and application to which it applies is provided in Table 5. These events are viewable as log files in the Audit Trail Viewer.

STEMvision™ Admins users may create audit trail reports using the **Audit Trail Viewer**. The viewer may be opened from the Windows Start menu. Click the “STEMvision” folder on the Start menu and select “Audit Trail Viewer”. The Audit Trail Viewer application window will open.

Table 5. Audit Trail Event Types

EVENT	WEL ID	EVENT DESCRIPTION	APPLICATION		
			Acquisition	Analyzer	Colony Marker
Application Started	1000	The application was started	✓	✓	✓
Application Stopped	1001	The application was closed	✓	✓	✓
File Created	1002	The application created a file. The name and location of the file is recorded, along with the file's checksum, if applicable.	✓	✓	✓
Plate Analyzing Environment	1007	Lists the Analyzer engine version and the serial ID of the STEMvision™ dongle		✓	
EOR Created	1004	A CFU Assay Report was created. Lists the type of report created and the sample information and analysis for which a report was created.		✓	
HL7 upload success	1005	Sample data were successfully sent to an HL7 server		✓	
Import Success	1006	The Analyzer application has successfully imported data edited in Colony Marker		✓	
Plate Imaging Parameters	1007	Lists the imaging parameters for an instance of image acquisition. This includes the STEMvision™ Unit ID, Plate ID, Barcode ID, Image Type, Exposure Time, Exposure Peak, and Focus Level.	✓		
EOR Approved	1008	A CFU Assay Report was signed by a user. Record of the user ID and type of approval (Technician or Lab Manager) as well as the user currently signed on to the STEMvision™ system.		✓	
Import Reset	2001	The pending Colony Marker modification data were reset		✓	
User Abort	2002	An acquisition or analysis process was aborted by the user. For the Analyzer log, this includes the analysis type and file aborted.	✓	✓	
File Error	3001	An error occurred when generating a file. Reports the application that generated the error, the file in which the error occurred, and the error message.	✓	✓	✓
EOR Error	3002	An error occurred when generating a CFU Assay Report. Identifies the error that occurred when attempting to generate a report.		✓	
File Validation Error	3003	The file that was attempted to open did not match the checksum recorded for the file		✓	✓
HL7 Upload Failed	3004	An error occurred while trying to connect to an HL7 server to upload sample data		✓	
Import Error	3005	Results failed to import from Colony Marker. The type of error that caused the import to fail is listed.			
Unknown Error	3100	An unidentified error has occurred	✓		

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Opening an Application Log

A directory containing the log files of interest may be selected. Application-specific log files are located in the C:\CFC\History folder for each of the STEMvision™ applications. For example, to generate a log report specific for Acquisition software, click the “Encrypted Log Directory” button and browse to the C:\CFC\History\Acquisition folder. Click the “Select Folder” button. All the log files present in the application folder will be queried and the event log compiled and shown in the log viewer window. The events are shown in a CSV format.

When a new folder is selected, the currently shown events are cleared, and events of the log files in the newly selected folder are shown. The events may also be cleared from the log viewer by clicking the “Clear Log” button.

Exporting an Application Log

The events of the log file may be copied into a spreadsheet, or saved as a CSV file for import into a spreadsheet.

To copy the log, select the events of interest in the log viewer window and copy to the Windows clipboard. This may then be pasted into a third party application as desired.

To save a log, click the Save Log button, and in the Save File As dialog box enter a folder path and desired log file name. Click the Save button to write the log data to the specified folder.

Creating an Audit Trail Report

Once the log file has been exported, it may be opened in a third party spreadsheet application. Many spreadsheet applications will parse the log data into the comma-separated columns. The column headings are equivalent for the logs of each of the STEMvision™ applications, and are shown in Table 6. An example workflow for generating an audit trail report for a specific application is shown in Figure 19.

Table 6. Column Headings of Exported Audit Trail Table

COLUMN NUMBER	COLUMN HEADING
1	Timestamp
2	Application
3	User ID
4	File Created
5	Assay Type
6	Checksum
7	Event Type
8	Event Extension Data **Plate Imaging Parameters**: Unit ID, Plate ID, Barcode, Image Type, Exposure Time, Exposure Peak, Focus **Plate Analyzing Environment**: Analyzer engine version, Dongle ID, Unit ID **Colony Marker**: Modified file

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Example: Find all STEMvision™ activities performed by the user “STEMvision” on a given date.

1. Open the Audit Trail Viewer and browse to the C:\CFC\History\Acquisition folder. Click the “Select Folder” button to generate the event log for the application.
2. Save the log file as “Acquisition Log” to a desired location.
3. Repeat for the Analyzer and Marker folder.
4. Import the saved log files into Microsoft® Excel or similar spreadsheet application. Insert a row at the top of the imported log and copy column headers from Table 6 into this.
5. Filter columns for desired date range and user name.

Timestamp	Applica	User ID	File Created	Assay Ty	Checksum	Event type	Event Extension Data	
2021-01-07 10:39:54	Analyzer	STEMvision				Application Started		
2021-01-07 10:39:57	Analyzer	STEMvision				Plate analyzing environme	Engine Ver:2.0	Client Ve
2021-01-07 10:41:17	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	F0980C6C3B8DD3F45998B58EC00C7BBE	File Created		
2021-01-07 10:41:17	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	4988635E8990A1A15BF5434EA11C913A	File Created		
2021-01-07 10:48:17	Analyzer	STEMvision				Plate analyzing environme	Engine Ver:2.0	Client Ve
2021-01-07 10:49:11	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	7D8AACDA4D55BFA359A8EB9834088272	File Created		
2021-01-07 10:49:11	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	08055F1A4A76250389DBFC62E6C74A62	File Created		
2021-01-07 10:50:12	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	831E7219DB2E0C9B73F841FDE1550279	File Created		
2021-01-07 10:50:12	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	B6AD43CE2993CFAF84901FA50291399C	File Created		
2021-01-07 10:51:20	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	0B9F5233D96AD1A3225176E1E1EF2D7C	File Created	Plate Imaging Paramete	
2021-01-07 10:51:20	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	5A6A16E4DA5E41A2162567199D2FA73C	File Created		
2021-01-07 10:52:16	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	6F5CC5ACE931CBA419AA2E27189E6AC0	File Created		
2021-01-07 10:52:16	Analyzer	STEMvision	C:\STEMvisionD	hCB14Day	E75B31B76A2DB4E8B3771B5530900B84	File Created		

Figure 19. Creating a Summary Audit Trail from the STEMvision™ Event Log

8.7.2 Windows Event Log (WEL)

The WEL provides an alternative method for generating STEMvision™ Application Log reports. In addition, the WEL is accessible to STEMvision™ Users and STEMvision™ Admins.

In order to generate a log report, open the Event Viewer from the Windows start menu. Click the “STEMvision” folder on the Start menu and select “Event Viewer”. The Windows Event Viewer application window will open (see Figure 20). From the Console Tree (left panel) of the Event Viewer, expand the “Applications and Services Logs” item and locate the STEMvision™ entry. Select the STEMvision™ entry to display the STEMvision™ application event log in the event (middle) panel of the Event Viewer.

By default, the event fields shown include the event level, timestamp, source application (STEMvision™), event ID, and task category (the STEMvision™ application that generated the event). Additional fields can be added by selecting “View” from the menu bar and clicking on “Add/Remove Columns”. If desired, select “User” from the available columns and add to the displayed columns. Similarly, unwanted columns can be removed from the event panel. When an event is selected, the details related to the event are displayed in the preview panel (see Figure 20). Events may be sorted by column simply by clicking the column header. Events may also be filtered for desired information. From the “Actions” panel on the right, click on “Filter Current Log”. The event log may be filtered by time, event level, event ID, user, and any keyword of interest. For the event IDs associated with each STEMvision™ application event, refer to Table 5.

Consult Microsoft® Windows® documentation for additional details.

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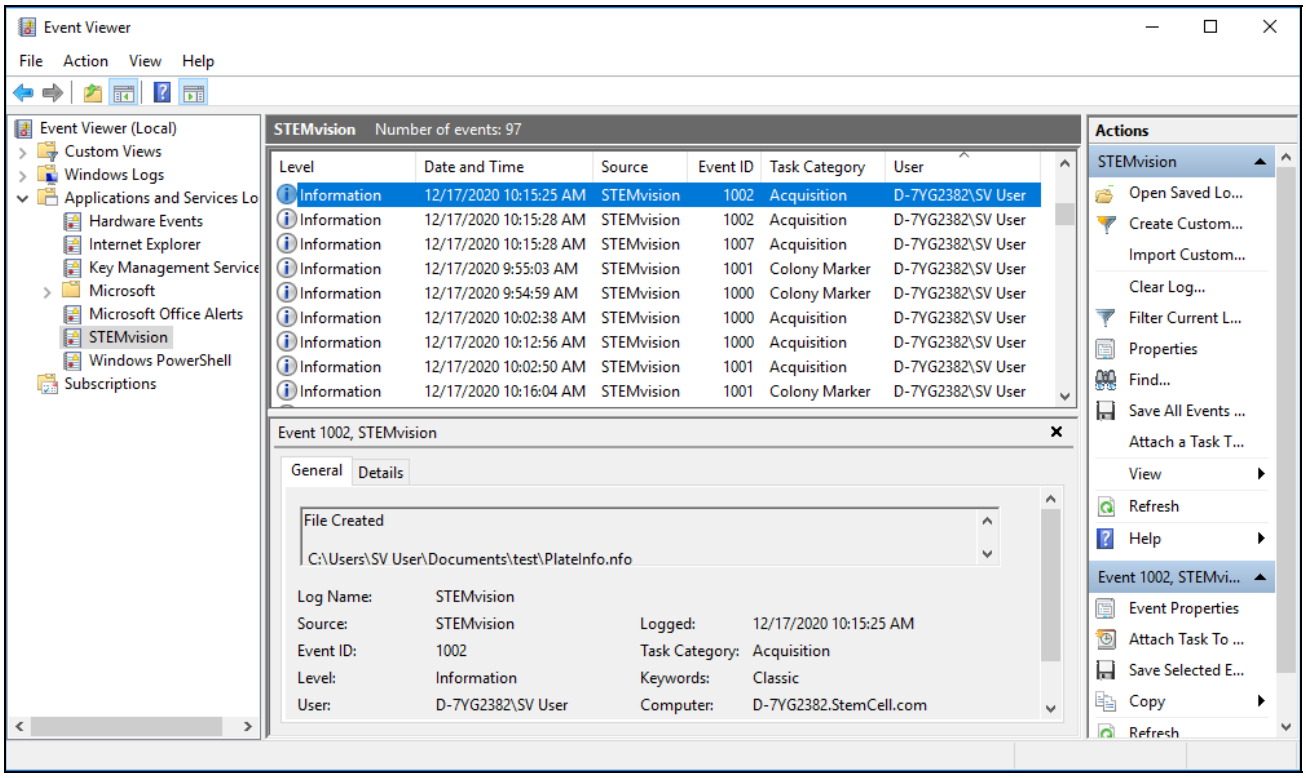

Figure 20. Event Viewer Application Window

8.7.3 Backing Up the Audit Trail

The STEMvision™ Application Log files reside in the C:\CFC\History folders for each application. The log files may be backed up by copying these folders to a backup location. If a log file is lost or corrupted, the backup copy can be viewed using the “Audit Trail Viewer” application as described in section 8.7.1.

The WEL for STEMvision™ is automatically archived when it reaches its maximum size (4 MB). The archived file is stored in the same location as the current event log with a filename of “Archive-STEMvision-YYY-MM-DD-HH-MM-SS-MSS.evtx”. Additionally, the event log can be saved manually to a backup location. In the Console Tree, expand “Applications and Services Logs” and right-click on the STEMvision™ entry. From the context menu that appears, click “Save All Events As…”. Save the log in the Windows native .evtx format to a backup location.

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9.0 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™.

9.1 Normal Maintenance

Turn off the STEMvision™ instrument when it will not be used overnight or for a significant period of time during the day (section 3.3). 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 10.0 to increase the lifetime of the STEMvision™ instrument.

9.2 Verification of Stage Positioning

The correct positioning of the STEMvision™ 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 (section 4.1).
3. Click the “Set Focus & Illumination” 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 will 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 21 (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 21 (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 21, 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.

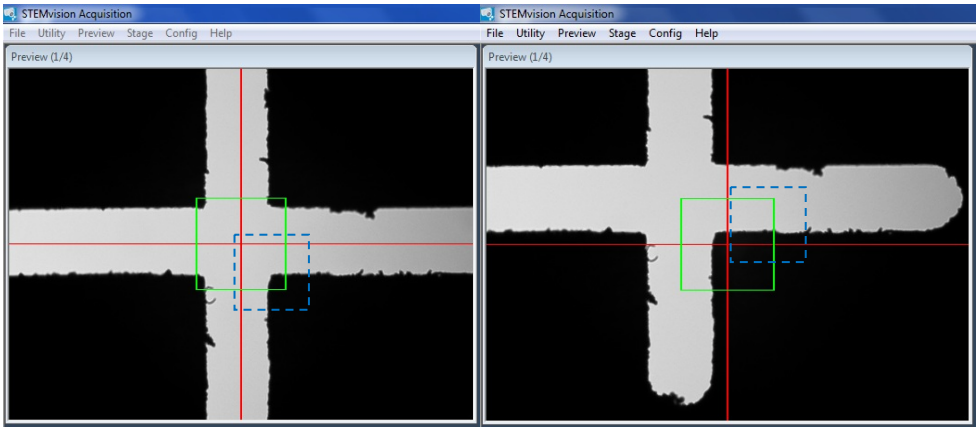

Figure 21. STEMvision™ Stage Position: Correct (left) and Incorrect (right) Positioning

Blue dashed square indicates the targeted area (center of cross).

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9.3 Cleaning an External Spill

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

9.4 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 decontamination 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 at techsupport@stemcell.com with a description of the failure so the appropriate corrective action can be determined.

9.5 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 at techsupport@stemcell.com with a description of the failure so the appropriate corrective action can be determined.

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10.0 Specifications

Capacity	One 6-well SmartDish™ plate at a time
Dimensions	478 mm W x 335 mm D x 347 mm H 18.82 in W x 13.19 in D x 13.66 in H
Weight	STEMvision™: 59 lb or 27 kg Computer: 23 lb or 10.5 kg
Power Requirements	100 – 240 V~, 50/60 Hz, 1.6 A Fuse 250V 2A Fast Blow
Optimal Operating Conditions	15 - 30°C 20 - 85% relative humidity Not specified for use inside an incubator Does not require placement in a biohazard safety cabinet Indoor use only Not to be used in a cold room
Storage and Shipping Conditions	-30°C to 50°C 10 - 90% relative humidity
Applied Standards	Certified to CAN/CSA STD 22.2 No. 61010-1-12 Conforms to UL STD 61010-1:2012 Conforms to IEC STD 61326-1:2012 / EN STD 61326-1:2013 FCC Part 15 Subpart B: 2014 ICES-003:2014
Meets all the Provisions of the Indicated Directives	2014/35/EU (low-voltage directive) and 2014/30/EU

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 European directives 2014/35/EU and 2004/108/EC. STEMvision™ meets the requirements of CAN/CSA C22.2 No. 61010-1-12 and UL standard No. 61010-1:2012 (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 back panel:

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™.

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11.0 Appendix 1: Examples of “Lab” and “Parent” Human CFU Assay Report Forms

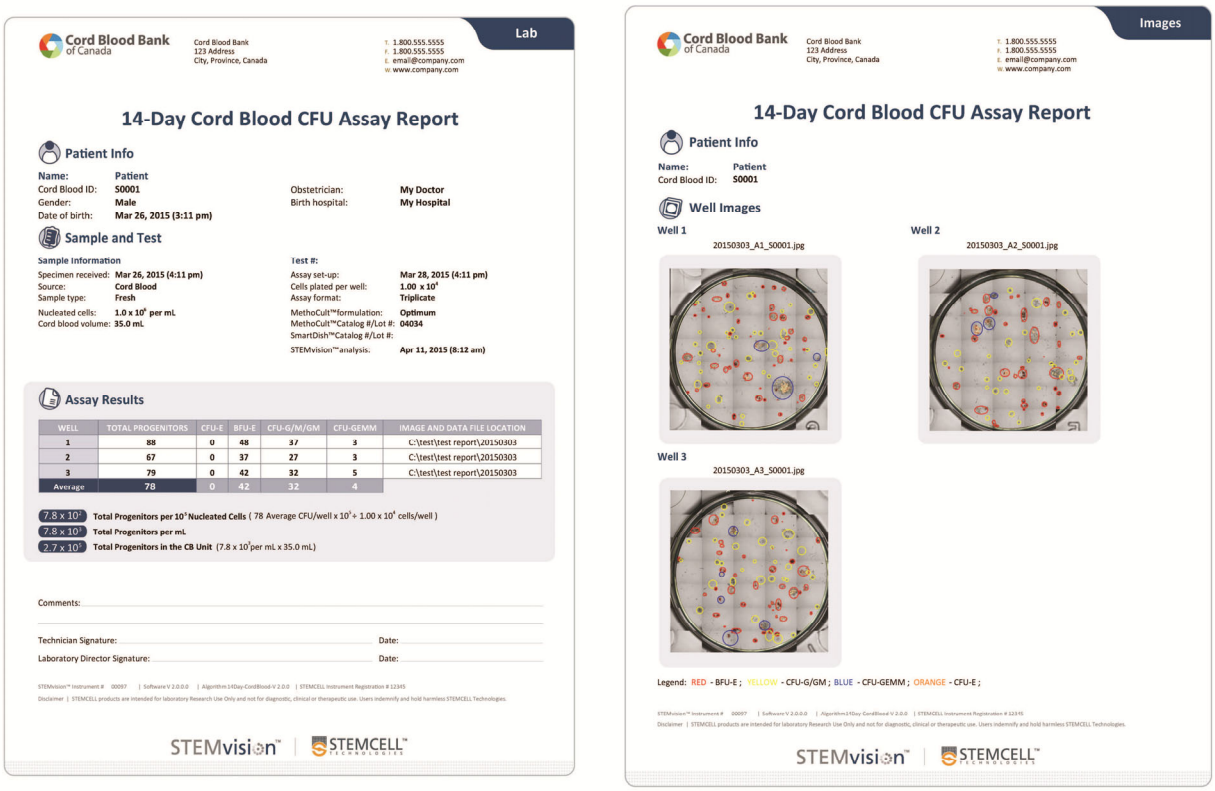

Figure 22. “Lab” Format of the Human CFU Assay Report Form for CB Cells

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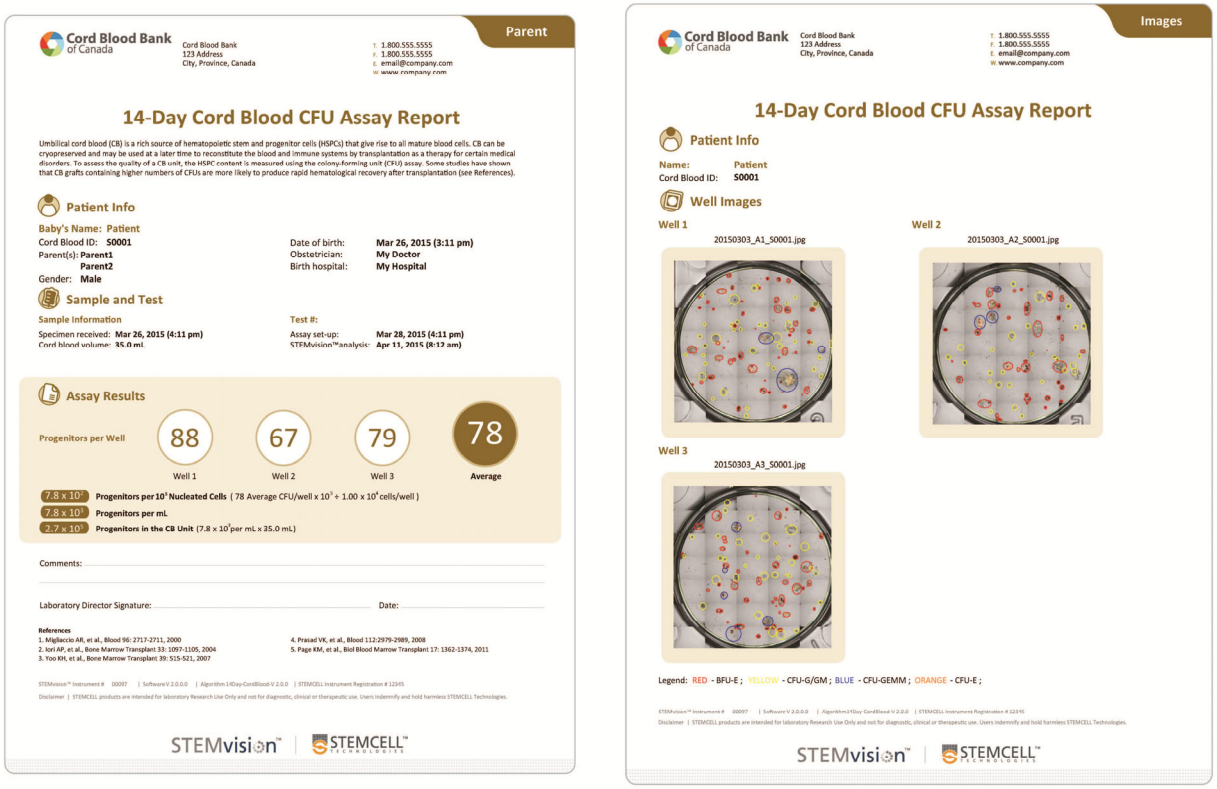

Figure 23. “Parent” Format of the Human CFU Assay Report Form for CB Cells

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TECHNICAL MANUAL

STEMvision™
Automated Colony-Forming Unit (CFU) Assay Reader

TOLL-FREE PHONE 1 800 667 0322
PHONE +1 604 877 0713
INFO@STEMCELL.COM
TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

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