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USER MANUAL

CELLOMETER® AUTO 2000

8001334 Rev G

For Research Use Only. Not for use in diagnostic procedures.

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Cellometer® Auto 2000 User Manual

8001334 Rev G

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Chapter 1. Introduction

This chapter presents introductory information to be reviewed *prior* to unpacking your Cellometer® Auto 2000 instrument. It includes a product overview, lists contents of shipping container, identifies symbols/abbreviations used on both the device label and instrument, and provides a summary of topics contained in this user manual.

AUTO 2000 OVERVIEW

Cellometer® Automated Cell Counters incorporate the basic principles of Imaging Cytometry traditionally used in manual cell counting via hemocytometer into a suite of instruments designed to simplify the cell counting process. By capturing multiple images of cells to be counted, users can adjust parameters based on cell morphology and perform reanalysis as necessary. Cellometer *Counting Chambers* are equivalent to all four corner squares of a hemocytometer and are disposable, thus saving time and effort, and eliminating any risk of cross-contamination.

Using both *Brightfield* and *Dual-Fluorescent* imaging in combination with pattern-recognition software, the Cellometer Auto 2000 quickly identifies cells and accurately calculates *Cell Count*, *Mean Diameter (micron)* and *Cell Concentration (cells/mL)*. Customizable cell type parameters are designed to assist with the declustering of clumpy cell lines. For cultured cells stained with trypan blue or fluorescent reagents, the Auto 2000 simultaneously calculates *Live/Dead* and *Total* cell counts, *Viability (%)*, and *Live* and *Total* cell concentrations, including a helpful graphic overlay used to highlight live and dead cells with color-coded outlines in the image being viewed.

The Auto 2000 is an all-in-one standalone instrument offering a large integrated touch screen that ensures simple operation and user-friendly visualization of cell morphology and counted cells. As the built-in computer can be attached to a network, count results and images can be stored to an external location (or on the included USB drive) and an Auto-Print feature can be enabled to generate a report for each counted sample. *Assays* allow users to customize counting parameters for specific cell types and a *Cell-size Analysis* tool generates histograms enabling optimization of cell diameter settings (e.g., to exclude debris or very large cells) during analysis of count results.

Intended Use

Intended use of the Auto 2000 is to count cell lines and primary cells (e.g., total nucleated cells, PBMCs, Splenocytes, stem cells, tumor/tissue digests, etc.) with the ability to determine cell count, viability and additional analysis using fluorescent reagents.

Personnel operating this instrument are encouraged to familiarize themselves with device controls and operation. Ensure that users can identify all components associated with the instrument, perform adequate adjustments and understand performance criteria. If issues are encountered, see *Troubleshooting and Instrument Messages* on page *85* to restore performance if instrument does not meet or exceed defined performance criteria.

⚠️ **WARNING: Use of controls or adjustments or performance of procedures other than those specified herein or by Revvity, Inc. may result in a hazardous process.**

CONTENTS OF SHIPPING CONTAINER

Cellometer Auto 2000 Instrument

Power Supply and **Power Cord** (Figure 1)

USB Drive (Figure 2)

    Contains **Cellometer Auto 2000** software.

Cellometer 2000 User Manual (PDF file on USB Drive)

Cellometer 2000 Quick Start Guide (PDF file on USB Drive
  and provided as printed copy with instrument)

Cellometer 2000 Focus Reference Guide

    Graphic sheet intended to help users adjust instrument focus.

Counting Chamber Slides (Figure 3)

    One box of 75 disposable slides; each slide contains
    two separate counting chambers.

Fluorescent Beads and **AO/PI Viability Reagent**

Figure 1. Power Supply/Cord

Figure 2. USB Drive

Figure 3. Counting Chamber Slides

ABOUT THIS USER MANUAL

This *Cellometer Auto 2000 User Manual* provides information on the following topics:

- Overview and Shipping Contents
- Potential Hazards and Safety Information
- Components, SN Label and Specifications
- Unpacking and Site Preparation
- Using Instrument for the First Time
- Key Software Screens
- Comparison of Viability Methods
- Slide and Sample Preparation
- Basic Counting and Analysis Workflow

- Assays and Customization Parameters
- Cell Types and Customization Parameters
- Defining User Counting/Saving Options
- Cleaning, Maintenance and Storage
- Troubleshooting and FAQs
- Contacting Support and Reporting Issues
- Consumable Slides, Reagents and Beads
- Warranty and License Details

The following *Precaution Signifiers* are used in conjunction with the ⚠ symbol in this user manual:

⚠ **IMPORTANT:** Note indicating that to skip or move past *<content_of_note>* may result in improper functionality of the instrument.

⚠ **CAUTION:** Note indicating that *<content_of_note>* may damage instrument to the point where it will no longer function as expected.

⚠ **WARNING: Note indicating that** *<content_of_note>* **may permanently damage instrument and cause personal injury or harm.**

GLOSSARY OF ABBREVIATIONS

The following abbreviations may appear on the shipping container, on the Cellometer Auto 2000 device label or in this user manual.

A	Amperes	**kW**	Kilowatt
AC	Alternating Current	**lb**	Pound
ANSI	American National Standards Institute	**PC**	Personal Computer
AO	Acridine Orange	**PI**	Propidium Iodide
API	Application Program Interface	**LED**	Light-emitting Diode
@	at	**MHz**	Megahertz
BR	Brightfield	**µg**	Microgram
°C	Degrees Celsius	**µL**	Microliter
cm	Centimeter	**µm**	Micron (Micrometer)
EU	European Union	**mL**	Milliliter
°F	Degrees Fahrenheit	**mm**	Millimeter
FCC	Federal Communications Commission	**ms**	Millisecond
FDA	Food and Drug Administration	**nm**	Nanometer
FL	Fluorescence	**OQ**	Operational Qualification
GUI	Graphical User Interface	**OSHA**	Occupational Safety and Health Administration
Hz	Hertz	**P/N**	Part Number
IFU	Information for Use	**SN**	Serial Number
in	Inch	**SW**	Software
IPA	Isopropyl Alcohol	**US**	United States
IQ	Installation Qualification	**USB**	Universal Serial Bus
kg	Kilogram	**V**	Volts
kHz	Kilohertz	**WEEE**	Waste Electrical and Electronic Equipment

GLOSSARY OF SYMBOLS

The following international symbols may appear on the shipping container, on the Cellometer Auto 2000 device label or in this user manual.

Symbol	Description	Symbol	Description	Symbol	Description
	Keep Dry. Located on Shipping Container		This End Up. Located on Shipping Container		Fragile, Handle with Care. Located on Shipping Container
	FCC Part 15 Supplier Declaration of Conformity.		European Conformity Mark. Located on Device SN Label		ISO 9001 Certified
	Polarity DC Power Connector. Located on Device SN Label		NRTL Safety Mark. Located on Device SN Label		On – Power Connection to Mains. Located on Instrument Power Switch
	Waste Electrical and Electronic Equipment Directive (WEEE). Located on Device SN Label	R-R-Nxb-Auto2000	Korean Certification for EMC Directives and Registration Number. Located on Device SN Label		Off – Power Disconnection from Mains. Located on Instrument Power Switch
SN	Serial Number. Located on Device SN Label	UK CA	UK Conformity Assessment Declaration of Conformity to UK Directives. Located on Device SN Label		Manufacturer. Located on Device SN Label

Chapter 2. Equipment Safety

As with any equipment that involves moving parts, there are potential hazards involved in the operation and maintenance of this instrument. This chapter identifies potential hazards and suggests precautions to avoid them.

POTENTIAL HAZARDS

This section describes instrument safety features designed to minimize potential hazards. Before using the system, familiarize yourself with this information.

> ⚠️ **WARNING: No modification of this equipment is allowed. Modification of equipment can result in improper operation causing possible injury.**
>
> In the United States, the facility operating the instrument should follow all OSHA Manual lines and applicable ANSI standards for the safe use of this instrument.
>
> Customer and operator agree that it is their sole responsibility to fully understand and comply with local, state, and federal laws, rules and regulations in the use of this system.
>
> Cables and accessories not specified within the instructions for use with this instrument are not authorized. Using other cables and/or accessories may adversely impact safety and performance.

Note: The Auto 2000 instrument should not be used adjacent to or stacked with other equipment unless specified by Revvity. If the system must be used adjacent to or stacked with other equipment, then observe the instrument in its configuration to verify instrument operation is normal and functions as expected.

Electrical Hazard

No part of the exterior housing should be removed. Do *not* open the instrument cover. For assistance, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

> ⚠️ **WARNING: To avoid the risk of electrical shock, this equipment must only be connected to a grounded electrical outlet.**
>
> **In addition, ensure electrical supply plug is not obstructed and can be reached by users to disconnect the device if necessary.**

Instrument System Hazard

Read the instructions, warnings and cautions provided with the instrument before using.

WARNING: Inspect instruments and cables for breaks, cracks, nicks and other damage before every use. This may be done visually under magnification or with a high voltage insulation testing device. If damaged, do *not* use. Damaged instruments or cables may result in injury to the user.

CAUTION: The instrument is designed to accept only one slide at a time. Do *not* attempt to load more than one slide into the sample slot. Doing so will cause an error and could damage the instrument.

CAUTION: Do *not* stack equipment on top of the Instrument or place the Instrument on top of electrical equipment. This is an unstable configuration and does not allow for adequate cooling.

In addition, it is recommended that as much distance as possible be provided between the instrument and any equipment emitting a high vibration signature. High levels of vibration may affect clarity of the image being viewed.

WARNING: Do *not* remove the instrument cover. For assistance, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Servicing Hazard

No one other than Revvity-authorized personnel may service inside the protective cover of the Cellometer Auto 2000 instrument system.

CAUTION: Do not pull the system by the connectors. Toppling of the system or causing damage to the system may result in the instrument no longer functioning as expected.

SAFETY INFORMATION

Personnel operating and maintaining the Auto 2000 instrument should be familiar with the safety information included in this section.

Safety Protocols

Revvity assumes no liability whatsoever for any damage, loss or injury resulting from an application of a product that is not in strict accordance with the instructions provided with the product. Revvity also assumes no liability for any damage or injury arising as a result of operator error or mistake, including, but not limited to, injury arising from operator's lack of qualification or as a result of errors or mistakes committed by such operator.

Read all installation and operation instructions contained in this user manual thoroughly before connecting the Auto 2000 instrument to the main power connection *prior* to use. The Auto 2000 must be set, regulated and used in accordance with the instructions outlined in this user manual. Failure to observe safety warnings and precautions may present a risk.

Only individuals with appropriate safety training and knowledge should operate, assist in the operation of, or perform cleaning and routine maintenance of this instrument. Only the operator should be responsible for system controls during a procedure.

Safety Features

The Auto 2000 Instrument offers several safety features to prevent its misuse or unintentional activation. All personnel who operate the system should be familiar with these safety features.

For assistance, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Federal Communications Commission

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.

ENVIRONMENTAL REQUIREMENTS

Environmental requirements for the intended operation of the Auto 2000 instrument are presented below.

– For Indoor Use Only

– Elevation: 0 to 2,000 m

– Temperature Range: 10 °C to 30 °C

– Relative Humidity: 0% to 90% RH, non-condensing

– Pollution degree: Degree 2

– MAINS supply voltage fluctuations up to ± 10% of the nominal voltage

IMPORTANT: If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

Chapter 3. Instrument Description

This chapter describes the Auto 2000 instrument components, device label and system specifications.

AUTO 2000 COMPONENTS

Auto Sample Slot: Counting chamber slides are inserted into this slot for viewing (accepts only one slide at a time).

Power Switch: Controls power going to the instrument. Button displays *Orange* to indicate power is *OFF* (in *Standby* mode); displays *Blue* when power is *ON* (in *Run* mode).

USB 2.0 Ports: Ports used to connect a USB device (e.g., thumb drive, keyboard, mouse or printer).

Ethernet Port: Port used to connect a network or internet cable to access the Auto 2000.

Power Cord Connector Input: Port used to connect the power supply to the Auto 2000.

Device Label: Lists the instrument serial number (SN), model, manufacturer and input power requirement.

DEVICE SN LABEL
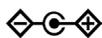
◇-C-◇	Polarity DC Power Connector
UKCA	UK Conformity Assessment Declaration of Conformity to UK Directives
KC R-R-Nxb-Auto2000	Korean Certification for EMC Directives and Registration Number
TÜVRheinland	NRTL Safety Mark
CE	European Conformity Mark
☒	Waste Electrical and Electronic Equipment Directive (WEEE)

INSTRUMENT SPECIFICATIONS

The Auto 2000 is your all-in-one automated cell viability counter. Using both *Brightfield* and *Dual-Fluorescent* imaging in combination with pattern-recognition software, it quickly and accurately identifies and counts cells. Results are automatically calculated and include cell counts, concentration, diameter and % viability.

	Auto 2000 Cell Viability Counter	
Channels	Brightfield and Fluorescent (FL1 - *Green* and FL2 - *Red*)	
Fluorescent Optics	Excitation / Emission: 470 nm / 535 nm *Example Fluorophores:* - Acridine Orange (+DNA) - Calcein AM - CFDA - SYTO® 9 - SYTO® 13	Excitation / Emission: 540 nm / 605 nm *Example Fluorophores:* - Propidium Iodide - Ethidium Bromide
Commonly Used Compatible Dyes	AO/PI, Trypan Blue	
Counting Speed (Per Sample)	<30 sec	
Volume (Per Chamber)	20 µL	
Cell Size/Diameter Range	5 – 300* µm	
Concentration Range	$1x10^5 – 1x10^7$	
Software	Cellometer Auto Pattern-recognition software to quickly and accurately decluster, identify and count individual cells	
Weight	26 lbs (11.8 kg)	
Width	11.1" (28.2 cm)	
Depth	12.8" (32.4 cm)	
Height	13.4" (34.0 cm)	
Input to Power Adapter	100-240 VAC, 50/60 Hz, 1.5A	
Output to Instrument	12 VDC, 7.5A	

*Cellometer CHT4-PD300 Slides are required for cells > 80 microns in diameter.

TECHNICAL INFORMATION

The following table presents Auto 2000 technical information including integrated computer and camera details.

	Auto 2000 Cell Viability Counter
Environmental Requirements	Typical biology lab environment.
Display	10.4", 1024 x 768 TFT with LED back light
Touch Screen	4-Pin resistive with controller 10.4" format
Processor	Intel® J1900
Memory	RAM Memory: 4 GB DDR3 Hard Drive Size: 500 GB SSD
Operating System	Windows 10 loT
External Connectors	2 USB 2.0 1 Ethernet port 1 Power Input Plug
External Button	On/Off Two Color Button. Orange: Standby | Blue: Run
Camera	½ " Interline 1.4 MP Monochrome Pixel size: 4.65 µm square
Circulation and Cooling	Allow at least 6 inches of unobstructed air circulation around vents on the instrument while in use to ensure adequate cooling.

Computer requirements indicated above (i.e., *Processor*, *Memory* and *Operating System*) represent minimum requirements applicable to any computer used to run the Auto 2000 software in *Data Analysis* mode for reanalysis of captured sample images.

DECLARATION OF CONFORMITY

Cellometer Auto 2000 conforms to appropriate country standards and governing regulations as listed in the *Declaration of Conformity*. To request the Auto 2000 *Declaration of Conformity*, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

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Chapter 4. Unpacking and Site Preparation

This chapter presents site preparation facility requirements for setting up the Auto 2000 instrument and transporting instructions if the instrument must be moved to another location.

UNPACKING/SETTING UP THE INSTRUMENT

Congratulations on your purchase of one of the best declustering cell counters available! Please ensure you have secured the space required to set up your new Cellometer Auto 2000 (see *Facility Requirements* on page *14*).

For assistance in setting up the instrument, visit the Cellometer Auto 2000 page on our website for a training video on unboxing and getting started.

Our staff would be happy to help you set up your Cellometer Auto 2000. For assistance, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

1. Inspect the package to ensure no damage has occurred during shipping, if applicable. ***Contact Support if damage has visibly affected the instrument.***
2. Ensure the box is facing up (i.e., *This End Up* symbol is facing in the right direction). If not, carefully turn the box right side up. *Box will weigh approximately 30 lbs (13.6 kg).*
3. Open the outer box.
4. Remove protective packaging and the inner box.
5. Open the secondary box to remove the Power Cord and USB Drive.
6. Remove the Auto 2000 and place it in the prepared area (see *Facility Requirements* on page *14*).
7. Remove the protective seals around the Auto 2000.
8. Insert the Power Cord Connector into the Auto 2000.
9. Plug the Power Cord into a surge protector (recommended) or power outlet.
10. Turn the Auto 2000 Power Switch to the ON position. The system is now ready for use.

Auto 2000 IQ/OQ Validation

Revvity has designed an *Installation Qualification/Operation Qualification (IQ/OQ)* validation specifically for the Auto 2000. Our experienced Support team is available to assist your organization during set up of the instrument and while performing the IQ/OQ validation. Contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

SITE PREPARATION

Facility Requirements

Instrument must be plugged directly into a surge protector (recommended) or power outlet. Ensure all cables are free from kinks or tangles *prior* to starting the Auto 2000.

Due to how the instrument is used with liquids, the instrument should be placed on a level surface.

Keep the area around the Auto 2000 clean between, during and post operation.

Electrical requirements for the Auto 2000 are as follows:

- 1.5 AMP service
- Wall receptacle voltage between 100 to 240 VAC
- 50/60 Hz

Note: Voltage requirements vary per region. For international customers, ensure that Power Cord meets local regulations or use a suitable replacement.

Note: The instrument can be disconnected from the mains by disconnecting the Power Cord from the mains plug or appliance coupler.

⚠️ **CAUTION:** Do *not* position the device so that it is difficult to disconnect from power main.

Transporting the Instrument

Prior to transporting the instrument by vehicle, disconnect the main from the wall and package all accessories and consumables individually and safely, ensuring cables are also protectively wrapped. Refer to instrument dimensions to ensure vehicle to be used for transporting meets these requirements.

When the instrument is loaded into the vehicle, it should be secured in such a way to prevent the instrument or components from shifting during transport.

⚠️ **WARNING: Care should be taken while moving or transporting the instrument to prevent damage to the instrument and/or possible injury.**

Disposal of Waste Electrical and Electronic Equipment

To comply with European Commission Directive on Waste Electrical and Electronic Equipment (WEEE) and other country and state regulations, do *not* dispose of this equipment in any location other than designated waste locations. For information regarding proper product disposal, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Chapter 5. Operation

This chapter describes the steps to be performed when using the Auto 2000 instrument for the first time. As the Auto 2000 is a touch-screen device, tap gently on screen elements (e.g., icons or buttons) using a finger or stylus to make software graphical user interface (GUI) selections. *As an alternative, a USB mouse may be connected.*

⚠️ **WARNING: Never place a foreign object inside the Auto Sample Slot (which is designed to accept only one slide at a time). Should anything get stuck or spill inside the sample slot, power OFF the instrument and contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com**

USING INSTRUMENT FOR THE FIRST TIME

Starting the Software

Power ON the instrument. The Auto 2000 software graphical user interface (GUI) will load automatically.

Taking a Background Image

After initial setup of the instrument, you must take a background image of the system by itself (without the counting chamber slide inserted) to normalize the cell counter. Unless the instrument is moved to a new location or image quality has degraded significantly, there is generally no need to take another background image.

1. Tap the **Settings** icon located on the bottom panel of the Home screen to edit instrument settings.

2. In the *Instrument Settings* area, tap the **Take Background Image** icon.

3. Remove the slide from the instrument, if applicable. Tap **Continue** in response to pop-up message to confirm Auto Sample Slot is empty.

4. The system takes the background image and displays a pop-up indicating when the image has been saved. Tap **Continue**.

5. Tap the **Preview** icon located on the bottom panel of the Home screen.
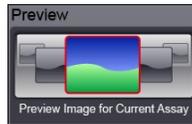
6. Confirm that the background is a uniform gray color.
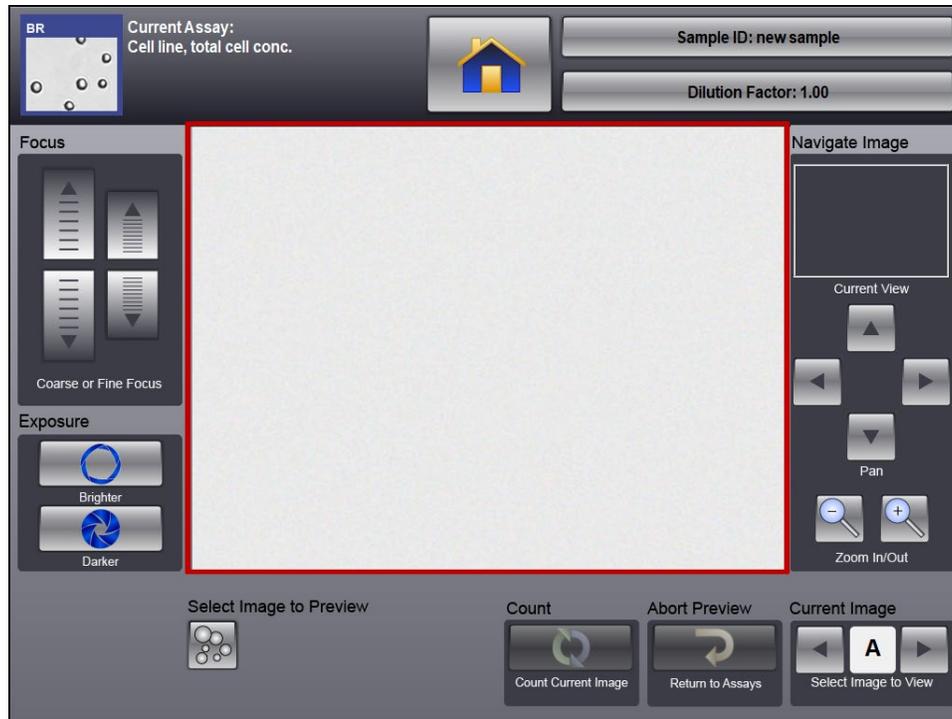
    If there is any discoloration (e.g., light/dark areas) of the image, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

7. Tap the **Home** icon located at the top of the screen to begin counting samples.

Chapter 6. Software GUI Screens

The Auto 2000 software graphical user interface (GUI) is pre-installed on the instrument and offers the fastest automated cell counting on the market. This chapter presents an overview of key screens.

Note: This instrument is a touch-screen device enabling users to interact directly with the GUI by tapping gently on screen elements (e.g., icons or buttons) using a finger or stylus. As an alternative, a USB mouse may be connected.

HOME SCREEN

The *Home Screen* (also referred to as the main screen) in the Auto 2000 software is described below.
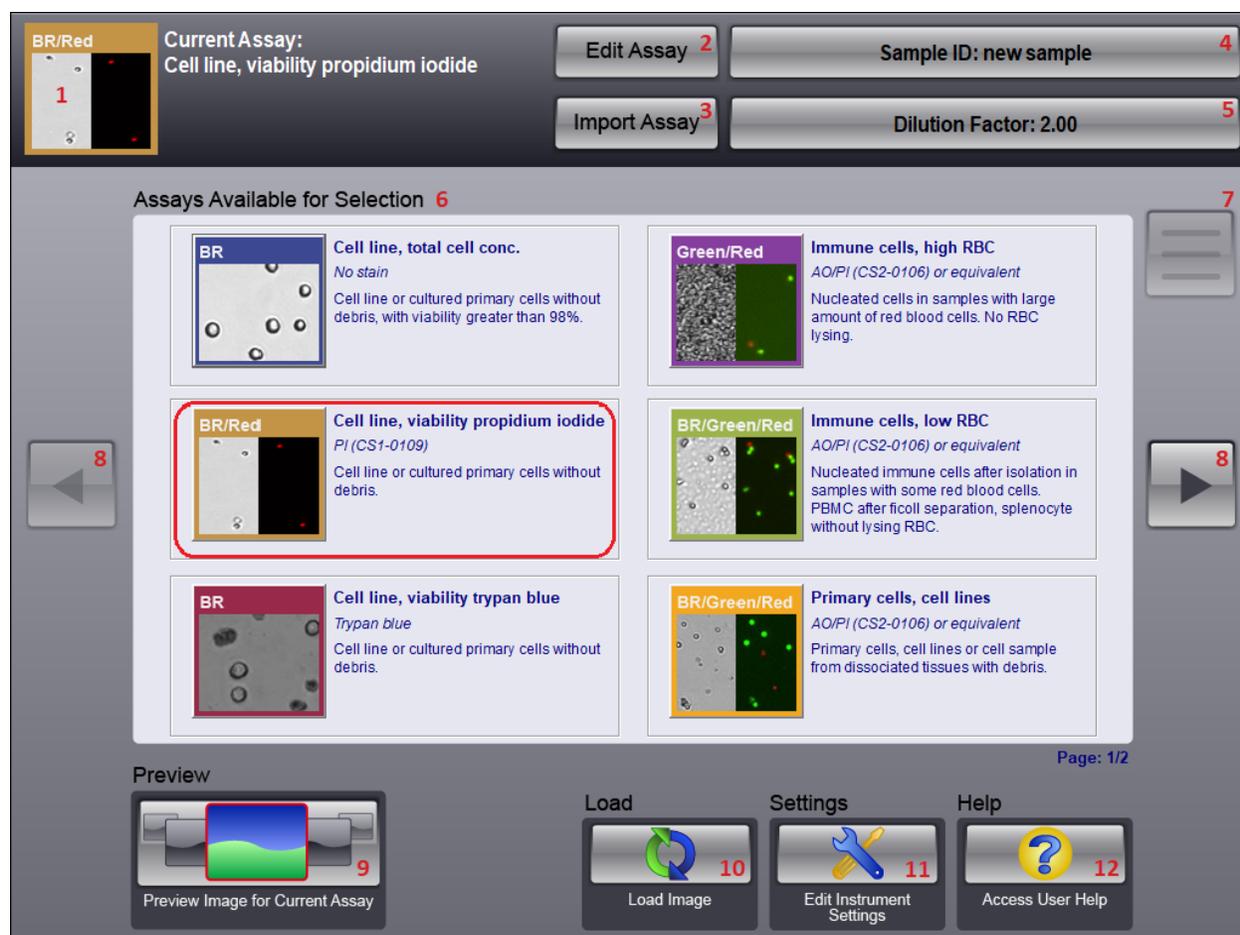
1 **Current Assay Indicator** – Displays assay that is currently selected for analyzing the sample. *The current assay is highlighted in the Assays Available for Selection list with a red outline.*

2 **Edit Assay Button** – Enables users to customize the currently selected assay and cell type parameters.

3   **Import Assay Button** – Enables users to import and export assays from/to an external library.

4   **Sample ID Input Field** – Allows entry of a unique sample identifier.

5   **Dilution Factor Input Field** – Allows entry of final dilution factor for calculating accurate concentration.

6   **Assays Available for Selection List** – Displays all assays available for analyzing samples. *The current assay is highlighted in this list with a red outline.*

7   **Last Count Results Icon** – When enabled, displays last available count results.

8   **Left/Right Arrows** – If the *Assays Available for Selection List* extends beyond a single screen, enables users to scroll left or right to view all assays.

9   **Preview Icon** – Displays a preview image for the currently selected assay.

10 **Load Icon** – Loads previously saved sample images for analysis.

11 **Settings Icon** – Enables users to edit instrument and graphical user interface (GUI) settings.

12 **Help Icon** – Provides access to online Help contents and resources, instructions for submitting a support ticket, and Revvity contact information.

PREVIEW SCREEN

Tapping the **Preview** icon from the Home screen allows users to preview images for the current assay.
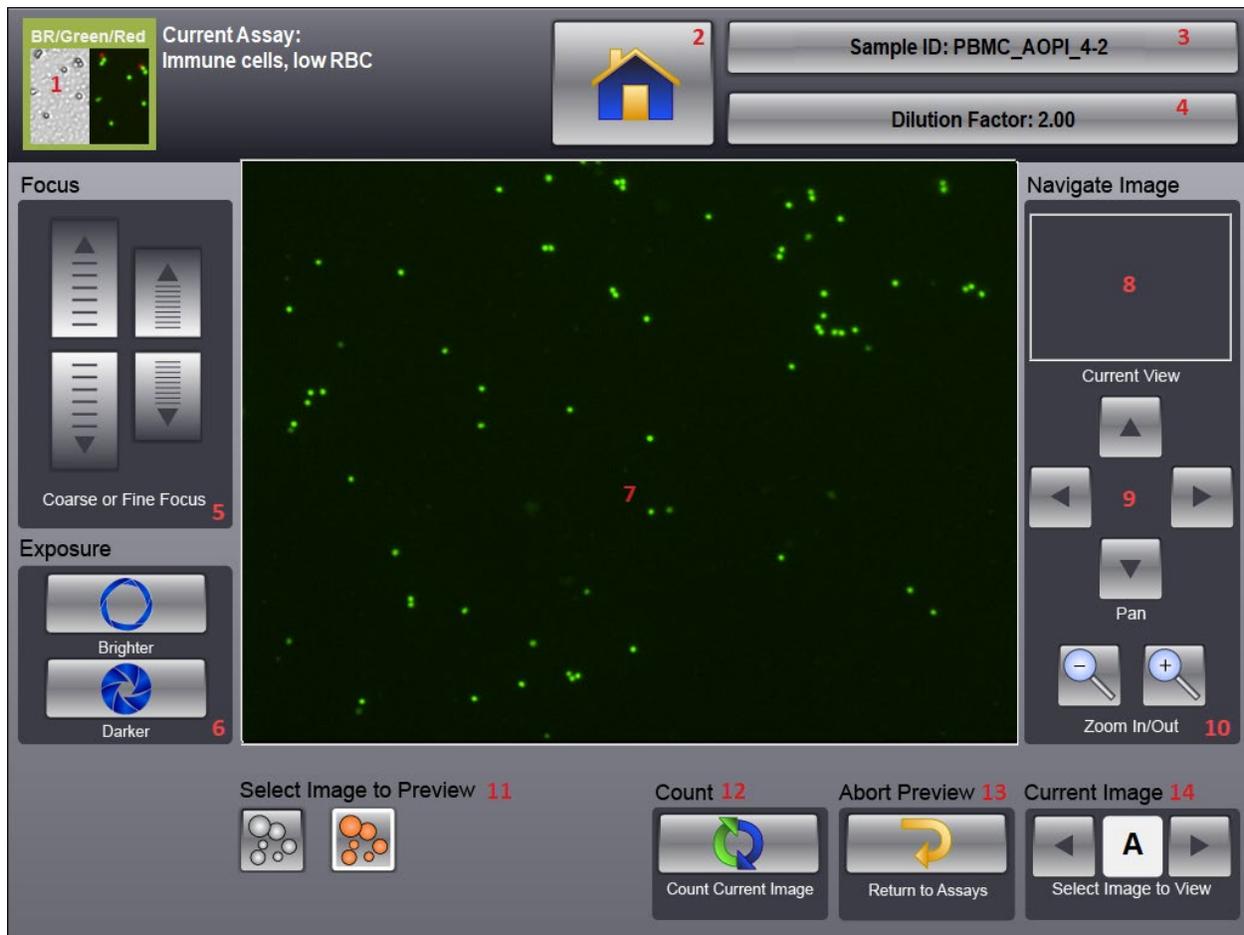
1. **Current Assay Indicator** – Displays assay that is currently selected for analyzing the sample.

2. **Home Icon** – Returns to Home screen for assay selection.

3. **Sample ID Input Field** – Allows entry of a unique sample identifier.

4. **Dilution Factor Input Field** – Allows entry of a final dilution factor for calculating accurate concentration.

5. **Focus Control** – Refines fine and coarse focus adjustment of the current sample preview. *Not enabled if a saved image has been loaded for display.*

6. **Exposure Control** – If current assay uses fluorescent imaging, adjusts FL Exposure Time of the image being previewed. *Changes made using this control will not be saved for the assay. This control will not be enabled if a saved image has been loaded for display.*

7. **Viewing Pane** – Displays area of sample image as shown in the *Current View* for the *Current Image selected*. Use the *Current Image Control* to switch between available images.

8. **Navigate Image Control, Current View** – Displays a red rectangle indicating area of sample image being displayed. *As you move the Pan and Zoom In/Out controls, the Current View will change accordingly.*

9   **Navigate Image Control, Pan** – Moves the area of sample image being displayed (indicated by the red rectangle in *Current View*) in the direction of arrows selected.

10 **Navigate Image Control, Zoom In/Out** – Zooms in or out of sample image being displayed (indicated by the red rectangle in *Current View*).

11 **Select Image to Preview Icons** – Displays icons for images available to be previewed based on the *Imaging Mode* defined for the current assay. The following combinations are possible:

Indicates a brightfield image is available. *Imaging Mode for current assay is either 'Brightfield Only' or 'Trypan Blue'.*

Indicates a brightfield image and *Green* fluorescent image are available (using the same channel). The *Green FL* image will be displayed by default. *Imaging Mode for current assay is either 'Brightfield and Fluorescence' or 'Fluorescent Only'.*

Indicates a brightfield image and *Red* fluorescent image are available (using the same channel). The *Red FL* image will be displayed by default. *Imaging Mode for current assay is either 'Brightfield and Fluorescence' or 'Fluorescent Only'.*
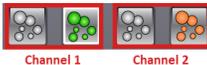
Indicates a brightfield image and *Green* fluorescent image (*Channel 1*) and a brightfield and *Red* fluorescent image (*Channel 2*) are available. The *Green FL* image will be displayed by default. *Imaging Mode for current assay is 'Two Fluorescent Stain' or 'Dual Stain'.*

Note: If fluorescent images are available, select the brightfield image associated with a fluorescent image to adjust instrument focus, then select the *Green* and/or *Red* icons to individually confirm focus and adjust FL Exposure Time. In addition, if both *Green* and *Red* fluorescent images are available, a slight offset exists between the *Brightfield 1* and *Brightfield 2* images due to the distance that the camera has to travel between the two filter sets.

12 **Count Icon** – Initiates counting of the sample.

13 **Abort Preview Icon** – Cancels the current sample preview and returns to the Home screen.

14 **Current Image Control** – Switches between images *A, B, C* and *D* of the sample. *Multiple images were taken during the counting process representing counting chamber fields of view.*

Counting Screen

Tapping the **Count** icon from the Preview screen initiates counting of the sample. Counts displayed in the *Count Total* area will vary based on the current assay (e.g., *Total* for assays containing live cells with no stain; *Total, Live and Dead* for assays stained with trypan blue).
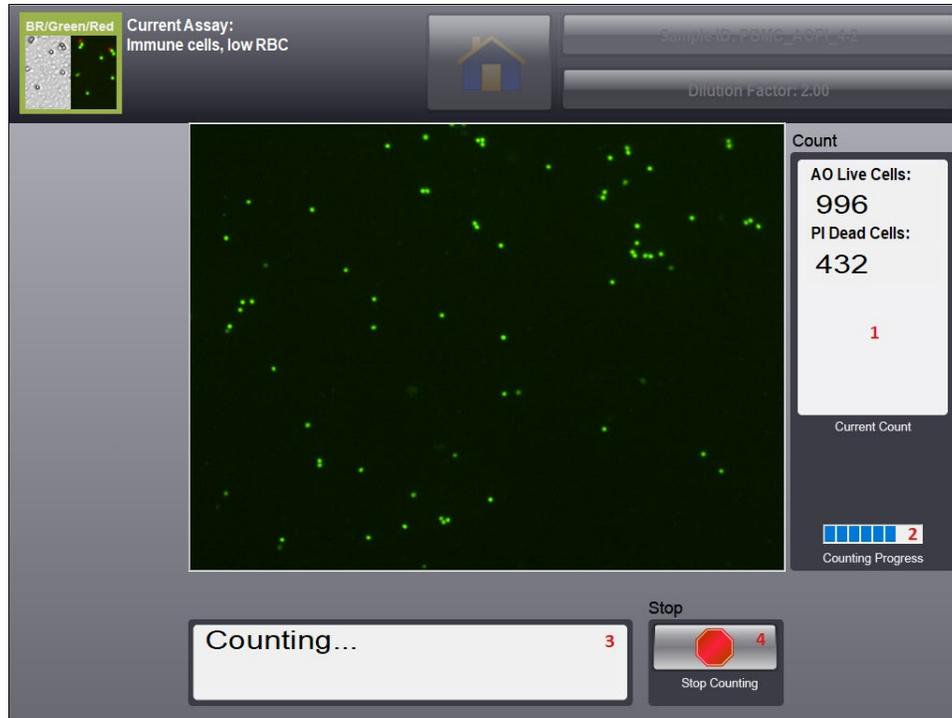
1 **Count Total** – Displays cell count as it accumulates throughout the counting process.

2 **Counting Progress Bar** – Indicates completion level of the counting process.

3 **Status Indicator** – Displays *Counting…* status indicating that count has been initiated.

4 **Stop** – Enables users to abort the counting process and return to the Home screen.

When the counting process is complete, the *Count Results* screen will automatically be displayed.

Count Results Screen

Count results displayed will vary based on the current assay (e.g., *Total Count*, *Concentration* and *Mean Diameter* for assays containing live cells with no stain; *Total/Live/Dead Count*, *Total/Live/Dead Concentration*, *Total/Live/Dead Mean Diameter* and *Viability* for assays stained with trypan blue). The format determining how these results are presented is defined by the *Result Template* selected for the assay which can be customized to meet the needs of your organization. See *Managing Templates* on page *55* for details.

1   **Current Assay Indicator** – Displays assay that is currently selected for analyzing the sample.

2   **Home Icon** – Returns to the Home screen.

3   **Sample ID Input Field** – Allows entry of a unique sample identifier.

4   **Dilution Factor Input Field** – Allows entry of a final dilution factor for calculating accurate concentration. *If dilution factor is modified after count is complete, results will be updated automatically*.

5   **Results Pane** – Displays cell count results for the sample. *The format used for presenting results is defined by the assay Result Template. See Managing Templates* on page *55 for details.*

6   **Details Icon** – Displays Count Details screen enabling users to view counted cells, save count data and images, and generate histogram based on cell size.

7   **Sample Icon** – Launches *Sample Adjustment* tool to calculate the sample adjustment needed to obtain desired concentration or target cell number. *See Calculate Sample Adjustment on page 45 for details.*

8   **Print Icon** – Sends the count results to a printer. *The format used for presenting results is defined by the assay Print Template. See Managing Templates* on page *55 for details.*

9   **Recount Icon** – Re-analyzes images for current scan and performs a new count. *Parameter settings may be updated between recounts.*

10   **Next Sample Icon** – Select to start preview of a new sample using the same Assay settings.

Count Details Screen

Tap the **Details** icon from the Count Results screen to view count result details. Data Options are available to analyze and save results.
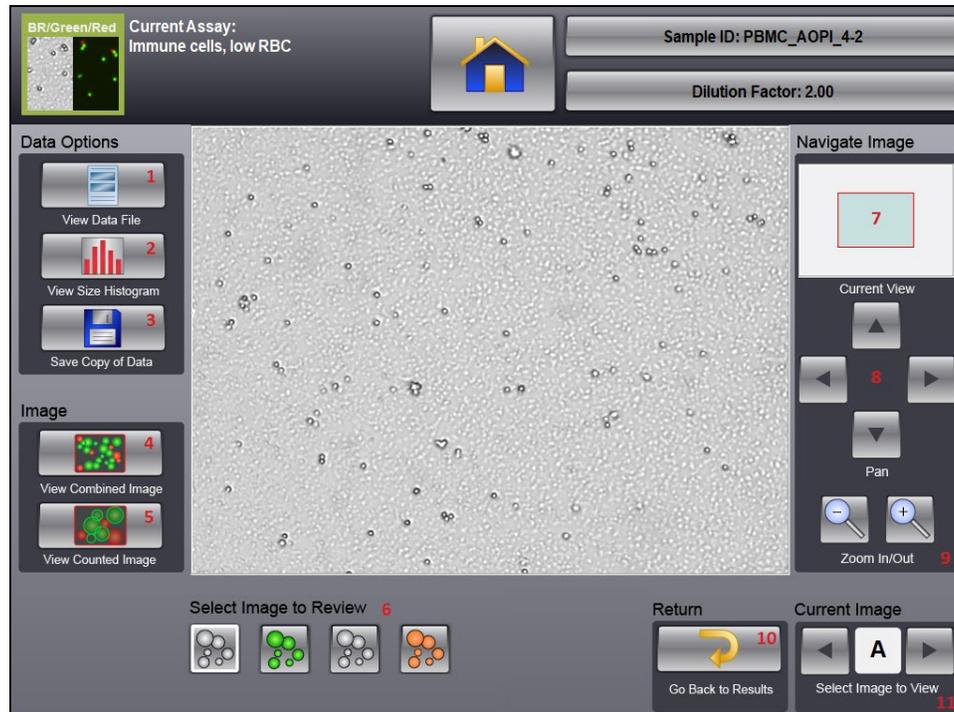
1   **View Data File Icon** – Displays data file of count results. Use the scroll bar at the bottom of the screen to view all columns and/or the **Print** icon to send the file to the printer. *Tap the **Save Copy of Data** icon to save current count results before viewing the data file if the Auto Save feature has not been enabled.*

2   **View Size Histogram Icon** – Launches *Cell Size Analysis* tool to display cell size data for your sample using a histogram plot. See *View Cell Size Analysis* on page *40* for details*.*

3   **Save Copy of Data Icon** – Select to save the current count results and images.

4   **View Combined Image** – Combines images taken through both channels. *Enabled only if both Red and Green fluorescent images are available.*

5   **View Counted Image** – Displays graphic overlay for the currently selected image highlighting counted cells (i.e., using green outlines for live cells, red outlines for dead cells and yellow outlines for cells *not* counted because they were larger than the specified cell diameter). *Although brightfield images associated with fluorescent images may not always display a counted graphic overlay based on the Imaging Mode defined for the current assay, they are necessary to counting algorithms used for the fluorescent images.*

6   **Select Image to Review Icons** – Displays images available to be reviewed based on the *Imaging Mode* defined for the current assay. The following combinations are possible:

      Indicates a brightfield image is available.

      Indicates a brightfield image and *Green* fluorescent image are available (using the same channel). *Brightfield image may not always display a counted graphic overlay depending on the counting algorithm used for the Imaging Mode.*

 Indicates a brightfield image and *Red* fluorescent image are available (using the same channel). *Brightfield image may not always display a counted graphic overlay depending on the counting algorithm used for the Imaging Mode.*
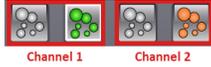
 Indicates a brightfield image and *Red* fluorescent image (through Channel 1) and a brightfield and *Green* fluorescent image (through Channel 2) are available. *Brightfield images may not always display a counted graphic overlay depending on the counting algorithm used for the Imaging Mode.*

Note: If both *Green* and *Red* fluorescent images are available, a slight offset exists between the *Brightfield 1* and *Brightfield 2* images due to the distance that the camera has to travel between the two filter sets.

7   **Navigate Image Control, Current View** – Displays a red rectangle indicating area of sample image being displayed. *As you move the Pan and Zoom In/Out controls, the Current View will change accordingly.*

8   **Navigate Image Control, Pan** – Moves the area of sample image being displayed (indicated by the red rectangle in *Current View*) in the direction of arrows selected.

9   **Navigate Image Control, Zoom In/Out** – Zooms in or out of sample image being displayed (indicated by the red rectangle in *Current View*).

10   **Return Icon** – Returns to the Count Results screen.

11   **Current Image Control** – Switches between images *A, B, C* and *D* of the sample. *Multiple images were taken during the counting process representing counting chamber fields of view.*

SAVING COUNT RESULTS

To save sample count results, tap the **Save Copy of Data** icon on the Count Details screen.

In the Save Options window, indicate the file types to be saved (e.g., text file, raw image .png file, .jpg image file and/or counted .jpg image file). *Only raw images can be loaded into the Auto 2000 for reanalysis or sent to Support when requesting assistance with optimization of assay/cell type parameters.*
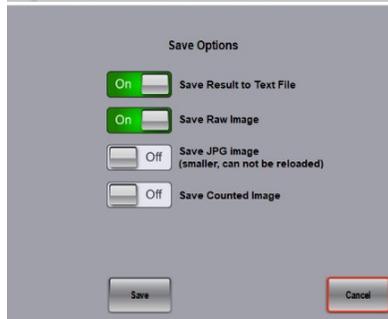
To save count result data to a text file, tap the **Save Results to Text File** option to display as *On*. Tap **Save** and respond to the confirmation prompt indicating the saved file location by tapping **Save** again.

Note: Count results are saved by default to a single .txt file in which rows (logged with a date/time stamp and Sample ID) are appended each time data is saved. You can set the default folder location and file name for this file stored in instrument settings. See *Defining Default Data File/Image Folder Locations* on page *80* for details.

To save image files, tap one or more file type options to display as *On*. Tap **Save** and then navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**. Click **Continue** in response to confirmation prompt.

If testing for cell viability, selecting this option displays a graphic overlay that shows a green outline around counted live cells and a red outline around counted dead cells.

VIEWING IMAGES AND COUNTED CELLS

When previewing images captured for a sample or reviewing count details, choose the images to display by tapping on an image icon. Images available to be previewed/reviewed are based on the *Imaging Mode* defined for the current assay.
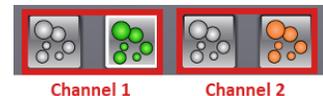
Note: For each fluorescent image taken, there will be an associated brightfield image to account for a slight offset between the FL1 and FL2 filters. *Brightfield images associated with fluorescent channels may not always display a counted graphic overlay depending on the counting algorithm used for the Imaging Mode.*

In addition, the **View Combined Image** (*enabled if using both FL filters*) and **View Counted Image** icons are available to refine the image being viewed by combining fluorescent images or adding a graphic overlay that highlights counted cells by circling them with color-coded outlines.

For example, the images shown below were taken using the *Two Fluorescent Stain* imaging mode. Images in each row were taken through the same FL filter, using both the FL 1 and FL 2 filters.
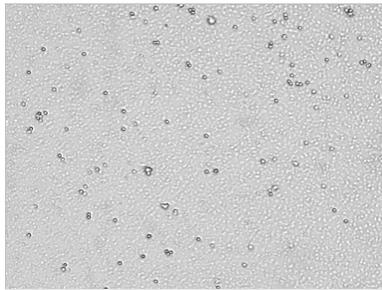
Preview of Brightfield 1 (FL 1 filter)
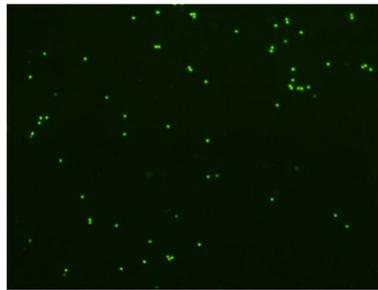
Preview of Green Fluorescent (FL 1)
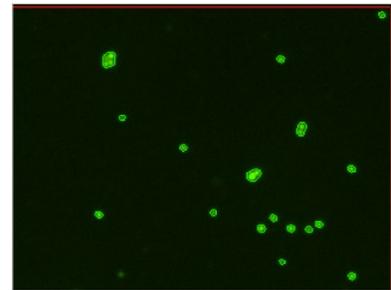
Green Fluorescent (FL 1), Counted
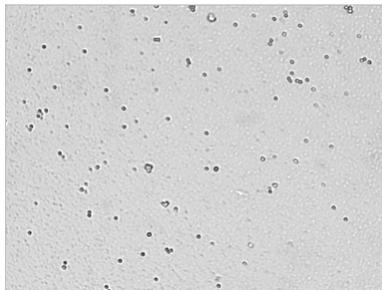
Preview of Brightfield 2 (FL 2 filter)
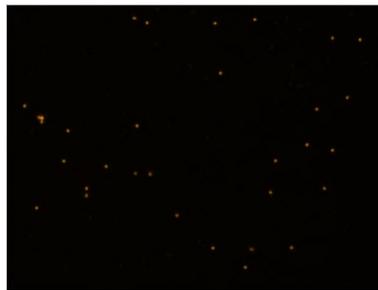
Preview of Red Fluorescent (FL 2)
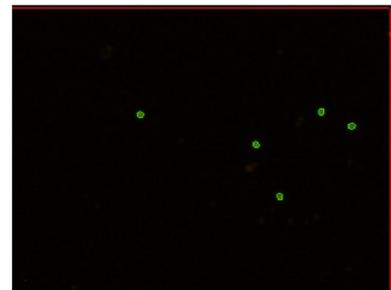
Red Fluorescent (FL 2), Counted
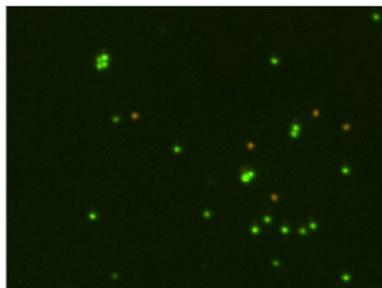
Red (FL 1) & Green (FL 2), Combined Image
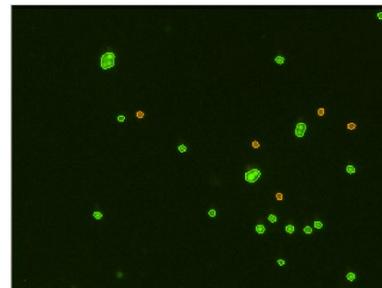

Combined Image, Counted

When viewing counted images, a color-coded graphic overlay is displayed for the image highlighting counted cells (i.e., green outlines for *counted* cells (or *live* cells depending on use of fluorescence), red outlines for *dead* cells and yellow outlines for cells *not* counted because they were larger than the specified cell diameter). This can help you identify if a cluster is being counted as one cell or two cells (as shown in the *Counted* images above).

Note: When viewing counted cells, a red line may appear along edges of the Viewing Pane identifying the outer perimeter of the counting area. *Positioning of this line will change as you navigate around edges of Current View pane using Pan and Zoom In/Out controls.* Any cells appearing on or outside of this red line are *not* counted.

IMPORT/EXPORT ASSAY SCREEN

Assays define image analysis parameters used in cell sample detection. Users can import assays from the built-in *Assay Library* installed with the Auto 2000 software or import custom assays from an external location.

Once a library is selected, you can highlight assays from the library (displayed in the pane on the left) and import them to appear in the *Assays Available for Selection* list available on the Home screen (displayed in the pane on the right). Assays selected to appear in the list can also be exported to create a new library.
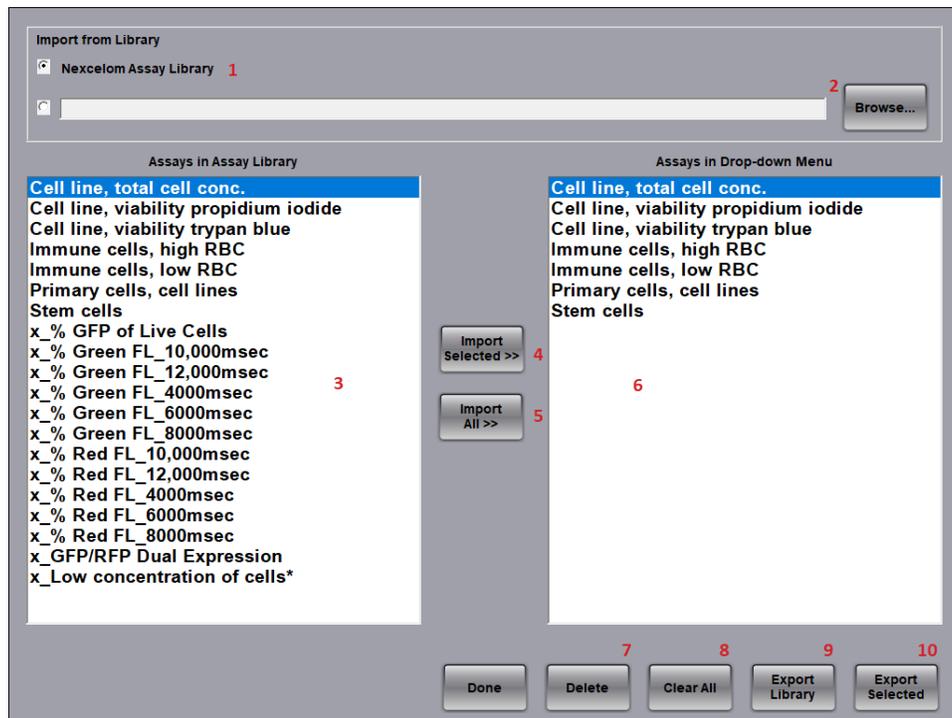
1	Selects **Nexcelom Assay Library** to display built-in assays in the *Assays in Assay Library* pane.
2	Selects a custom library to display available assays in the *Assays in Assay Library* pane. If no library name is displayed, tap the **Browse** button and navigate to an external Assay Library.
3	List of assays in the currently selected Assay Library.
4	Imports highlighted cell types (from the left pane) into the drop-down menu (right pane).
5	Imports all cell types (from the left pane) into the drop-down menu (right pane).
6	List of assays to be displayed in the *Assays Available for Selection* list on the Home screen.
7	Deletes any highlighted assay in the drop-down menu (displayed in the right pane).
8	Clears all assays in the drop-down menu (displayed in the right pane).
9	Exports all drop-down menu options (displayed in the right pane) to create an Assay Library.
10	Exports selected drop-down menu options (displayed in the right pane) to create an Assay Library

Importing Assays

Used to import assays from the built-in Assay Library or from external custom libraries. Individual assays can then be selected to appear in the *Assays Available for Selection* list displayed on the Home screen.

FROM BUILT-IN ASSAY LIBRARY

The built-in **Assay Library** is available by default and if selected in the Import/Export Assay screen, available assays are displayed in the *Assays in Assay Library* pane.

1. In the *Import from Library* section of the Import/Export Assays screen, ensure that the **Nexcelom Assay Library** radio button is selected.

2. In the *Assays in Assay Library* pane (displayed on the left), highlight an assay to be imported.

3. Tap **Import Highlighted >>** to import highlighted assay to appear in the *Assays in Drop-down Menu* pane (displayed on the right).

   Note: If all cell types are to be imported, select **Import All >>** followed by tapping **Copy All** in response to the confirmation prompt. Importing all assays is *not* recommended if the size of the library will make the *Assays Available for Selection* list in the Home screen difficult to use.

FROM CUSTOM LIBRARIES

1. In the *Import from Library* section of the Import/Export Assays screen, tap the custom Assay Library radio button to choose a custom library (if available) or tap **Browse** to navigate to folder containing a custom library and double tap on a library name to select it. *This text box will appear as blank if a custom Assay Library has not yet been selected.*

2. Ensure that assays from the selected library appear in the *Assays in Assay Library* pane.

3. In the *Assays in Assay Library* pane (displayed on the left), highlight an assay to be imported.

4. Tap **Import Highlighted >>** to import highlighted assay to appear in the *Assays in Drop-down Menu* pane (displayed on the right).

   Note: If all assays are to be imported, select **Import All >>** followed by tapping **Copy All** in response to the confirmation prompt. Importing all assays is *not* recommended if the size of the library will make the *Assays Available for Selection* list in the Home screen difficult to use.

Deleting and Clearing Assays

DELETING AN ASSAY

1. Tap the assay in the *Assays in Drop-Down Menu pane* (displayed on the right) to be deleted.

2. Tap the **Delete** button.

3. In response to the confirmation prompt, tap **Delete** to confirm the deletion.

4. Confirm that assay has been removed from the *Assays in Drop-Down Menu* pane.

Note: If assay deleted was from the built-in Assay Library, it can be re-imported if necessary. If assay was from a custom library or created using the Auto 2000, it may be permanently deleted unless assay was exported and saved prior to deletion.

CLEARING ALL ASSAYS

1. Confirm that all *Assays in Drop-Down Menu pane* (displayed on the right) are to be deleted.

2. Tap the **Clear All** button.

3. In response to the confirmation prompt, tap **Clear All** to confirm the deletion. Only the *Initial Assay Type* will remain as a default.

   Note: If assays cleared were from the built-in Assay Library, they can be re-imported if necessary. If assays were from a custom library or created using the Auto 2000, they may be permanently deleted unless assays were exported and saved prior to deletion.

Exporting Assays

EXPORTING ASSAY LIBRARY

1. Confirm that all assays in the *Assays in Drop-Down Menu pane* (displayed on the right) are to be exported.

2. Tap the **Export Library** button, select an **Available Drive** (displayed in bottom left corner of the screen) and then navigate to a network location by tapping folder icons.

3. Choose an existing file or tap **New File** to create a file. *If necessary, create a new folder before creating a file.*

4. Use the virtual keyboard to enter a file name and tap **Done**.

5. If prompted whether to *Lock the assays from future editing*, tap **Lock** or **Unlock**.

6. Tap **Continue** to return to the Import/Export Assay screen.

EXPORTING SELECTED ASSAY

1. Tap the assay in the *Assays in Drop-Down Menu pane* (displayed on the right) to be exported.

2. Tap the **Export Selected** button, select an **Available Drive** (displayed in bottom left corner of the screen) and then navigate to a network location by tapping folder icons.

3. Choose an existing file or tap **New File** to create a file. *If necessary, create a new folder before creating a file.*

4. Use the virtual keyboard to enter a new file name and tap **Done**.

5. Tap **Continue** in response to confirmation prompt indicating the name of the file to be saved and return to the Import/Export Assay screen.

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Chapter 7. Viability Methods

This chapter describes how to choose a viability staining method based on the cell sample and selected assay type.

EVALUATING VIABILITY METHODS

When evaluating viability methods, it is critically important to use a single aliquot from the stock cell culture to perform *all* testing. The cell sample should be evaluated for concentration on the Auto 2000 prior to staining.

If comparing the trypan blue and AO/PI methods, a portion of the sample should be stained with trypan blue and a portion should be stained with AO/PI. *Using samples from the same aliquot containing identical cell concentrations will result in a more accurate comparison of staining methods.*

Trypan Blue Viability

Brightfield imaging used in conjunction with *Trypan Blue Viability* may be used to determine the number, concentration and percentage of live cells for cell lines and cultured primary cells. Brightfield imaging with trypan blue viability is *not* recommended for samples containing debris, platelets or red blood cells. In these cases, fluorescence is required to accurately differentiate nucleated cells from platelets, red blood cells and debris.

PREPARING CELL SAMPLE FOR TRYPAN BLUE VIABILITY DETERMINATION

Cell concentrations of $1.0 \times 10^5 - 1.0 \times 10^7$ cells/mL can be analyzed on the Auto 2000, with a concentration of 1.0×10^6 cells/mL being optimal.

Invert the tube containing cells ten times (10x) and pipette up and down 10x to generate a homogeneous cell sample and reduce cell clumps. Do *not* shake or vortex the sample as this may damage cell membranes.

For viability measurement, stain cells by combining 50 μL of cell sample with 50 μL of a 0.2% trypan blue staining solution (for a final concentration of 0.1% trypan blue). Gently mix by pipetting up and down 10x.

AO/PI Viability

Dual-fluorescence methods have been developed to accurately determine nucleated cell concentration and viability in primary cell samples containing debris and non-nucleated cells, including platelets and red blood cells.

In the AO/PI viability method, acridine orange (AO) enters all cells and stains the DNA (causing nucleated cells to fluoresce *Green*) while propidium iodide (PI) enters dead cells with compromised membranes and stains the DNA, generating *Red* fluorescence in dead nucleated cells.

- Cells stained with *both* AO and PI fluoresce *Red* due to quenching.
- Live nucleated cells are easily identified in the *Green* fluorescence channel.
- Dead nucleated cells are easily identified in the *Red* fluorescence channel.

As a result, there is no interference from debris and non-nucleated cells when using the AO/PI viability method.
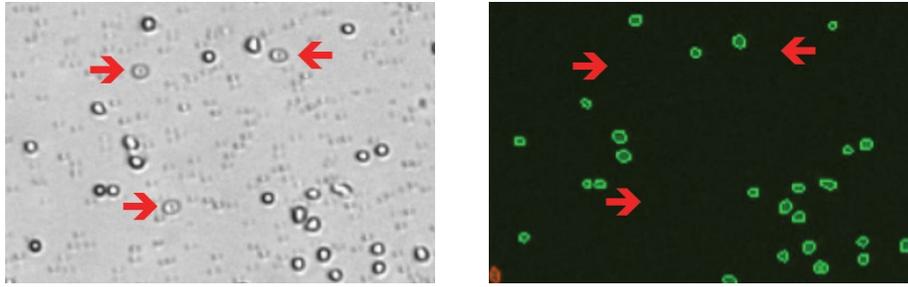
In images captured by the Auto 2000 using the AO/PI viability method, red blood cells seen in the brightfield image shown on the left (cells highlighted by arrows) are *not* seen in the fluorescent image shown on the right. Only nucleated cells are counted using the AO/PI staining method resulting in a more accurate total cell count and percent viability calculation.

PREPARING CELL SAMPLE FOR AO/PI VIABILITY DETERMINATION

Cell concentrations of $1.0 \times 10^5 - 1.0 \times 10^7$ cells/mL can be analyzed on the Auto 2000, with a concentration of 1.0×10^6 cells/mL being optimal.

Dilution or concentration of a cell sample may be required based on the initial concentration.

Invert the tube containing cells ten times (10x) and pipette up and down 10x to generate a homogeneous cell sample and reduce cell clumps. *Vortexing may help for some samples but may generate bubbles that make it difficult to pipette when working with small sample volumes.*

If necessary, dilute original sample with PBS. Stain cells by combining 20 µL of cell sample with 20 µL of AO/PI staining solution. For whole blood and other viscous samples, draw sample in and out of the pipette tip prior to transfer. Gently mix by pipetting up and down 10x before adding sample to counting chamber (CHT4).

The table below shows the recommended dilution when preparing cell samples for AO/PI viability analysis and the final *Dilution Factor* to enter into Auto 2000 software for a variety of sample types.

Sample Type	Preliminary Sample Dilution (with PBS)	Volume of Sample	Volume of AO/PI	Final Dilution Factor
Whole peripheral blood or cord blood	1:10	20 µL	20 µL	20
PBMCs following ficoll separation	*Not Required*	20 µL	20 µL	2
Mononuclear cells from processed bone marrow	*Not Required*	20 µL	20 µL	2
Tumor digest/Tissue digest	*Not Required*	20 µL	20 µL	2
Stem cells from CD34+ separation	*Not Required*	20 µL	20 µL	2

In the Auto 2000 Home screen, select an assay based on the primary cell type being analyzed. Enter a **Sample ID** and **Dilution Factor** (as shown in *Final Dilution Factor* column above), accounting for any other sample dilutions.

The following four assays are pre-optimized with specific counting parameters for the cell type(s) indicated below:

- *Immune Cells, High RBC* – Total nucleated cells or white blood cells from diluted peripheral blood, or cord blood
- *Immune Cells, Low RBC* – PBMCs, splenocytes and other primary cells following initial separation
- *Primary Cells, Cell lines* – Tumor digests and tissue digests
- *Stem Cells* – Stem cells

Chapter 8. Sample Preparation

This chapter describes how to prepare Cellometer counting chamber slides and load samples into the counting chambers on a slide. See *Counting Chamber Slides* on page *89* for a list of slides available for the Auto 2000.

PREPARING COUNTING CHAMBER SLIDES

1. For SD100 slides, remove protective film from both sides of the slide. *PD100 slides are peeled and ready to use.*

    Note: It may be difficult to peel protective film from slide. One method is to adhere tape to the top of film and then pull tape off (with film attached). Do *not* touch or write on the clear optical window areas of the slide.

Top of Slide

Bottom of Slide
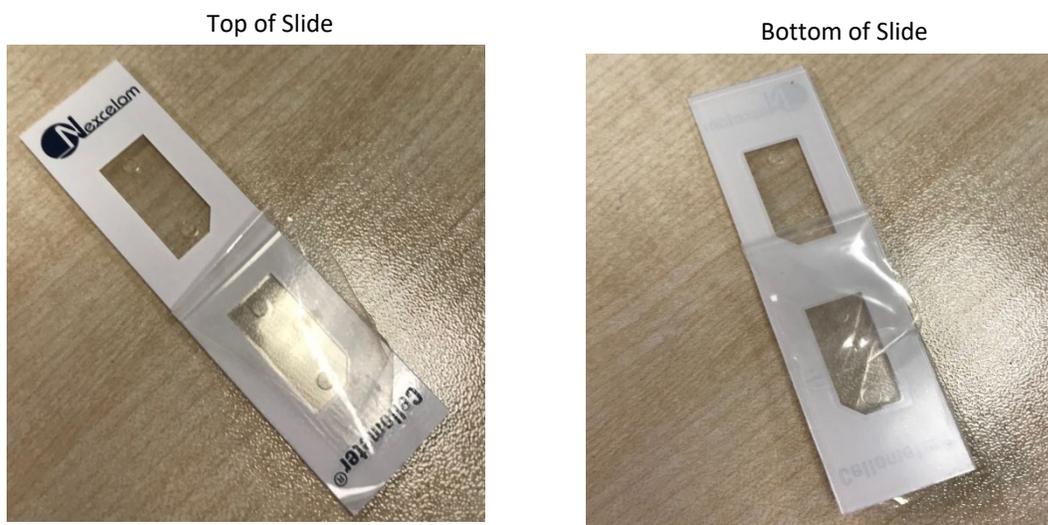
2. Place the Cellometer disposable counting chamber slide on a fresh Kimwipe.

3. To prepare two samples at once, label individual counting chambers (e.g., *Chamber 1* and *Chamber 2)* in the white margin of each chamber. Take care to ensure the clear portion of the counting chamber is not touched.

    Note: Both chambers of the slide do *not* need to be loaded with the same sample. Two different samples can be used on a single slide.

STAINING SOLUTION GUIDELINES

Use the following staining solution guidelines when preparing cell samples for analysis with the Auto 2000.

Stain Type	Use with Cell Sample	Dilution Factor
Trypan Blue (0.2%)	1:1	2
A0 (CS1-0108-5mL)	1:1	2
PI (CS1-0109-5mL)	1:1	2
AO/PI (CS2-0106-5mL)	1:1	2

See *Evaluating Viability Methods* on page *31* for details on how to choose a staining method based on the cell sample and selected assay type.

LOADING SAMPLES INTO COUNTING CHAMBERS

Cellometer counting chamber slides contain two independent chambers manufactured to a precisely controlled height. A cell suspension of 20 μL is pipetted into a counting chamber through its induction port.

1. To prepare the sample for counting, invert the tube containing your sample 10 times, then pipette up and down 10 times. This will help to evenly suspend cells. Do *not* shake sample as it may damage cell membranes.

    Note: If testing for cell viability (*optional*), stain the sample using a 1:1 ratio with 0.2% trypan blue (or other chosen viability stain) and mix the stain by pipetting up and down 10 times *before* performing Step 1, above.

    When preparing a sample to test for cell viability, ensure that the trypan blue stock concentration used is 0.2%. If stock concentration is 0.4%, it is recommended you dilute it with a balanced salt buffer (e.g., PBS) and filter diluted solution with a 0.2 micron filter before adding stain to your sample.

2. Immediately transfer 20 μL of sample into a counting chamber by pipetting directly onto its induction port. *There are two counting chambers per slide; each chamber may be loaded with a different sample.*

3. Hold the loaded slide in the white area and take care to stay away from the clear optical window.
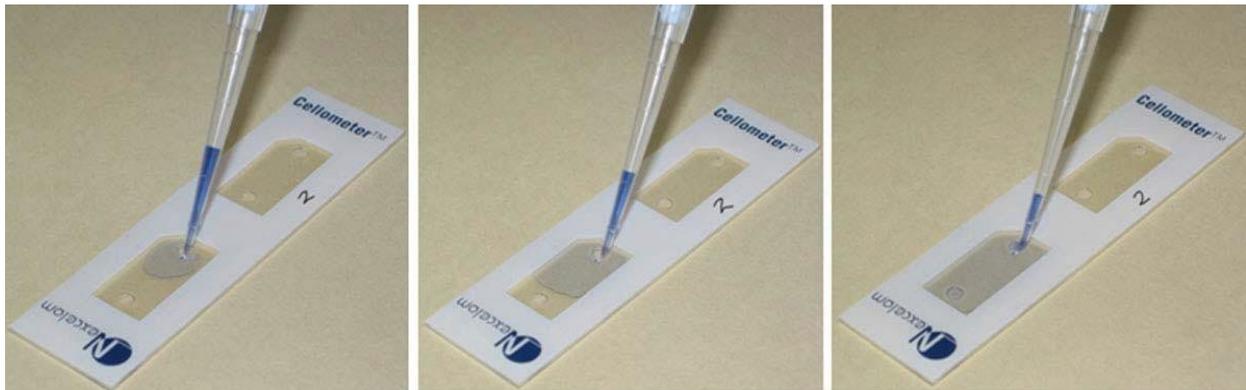
Load a counting chamber by touching pipette tip to the chamber's induction port and then slowly pipetting the entire 20 μL sample all at once. Capillary force automatically spreads the sample within the chamber. Each counting chamber can hold a separate sample as there is no mixing between the two individual chambers on a slide.

Chapter 9. Counting and Analysis Workflow

This chapter presents the basic workflow for counting cells/beads using the Auto 2000, including best practices and tips. Sample images are provided as a reference for many common cell types/beads.

PERFORM CELL COUNTING

1. Power ON the instrument.

2. Slowly Pipette 20 μL of sample into counting chamber. See *Preparing Counting Chamber Slides* on page *33*.

3. Load prepared slide into Auto 2000 by inserting the counting chamber that contains sample into Auto Sample Slot until touching the internal stop. *The chamber inside the instrument will be counted.*

   Note: If testing for cell viability, you must stain the sample before loading it into the slide chamber.


4. In the *Assays Available for Selection* area of the Home screen, tap gently on an assay to select it. *If assay list spans beyond the screen, arrows will be enabled on both the left and right sides to scroll back and forth through the list.*

5. Tap on the **Sample ID** input field and use the virtual keyboard to enter a sample name. Tap **Save**.

6. If necessary, tap on the **Dilution Factor** input field and change the value to indicate the final dilution factor for the sample. Tap **Save**.

7. Tap the **Preview** icon to view images of the sample.

8. Select the brightfield image to preview. *Two brightfield images (Brightfield 1 and Brightfield 2) may be available based on Imaging Mode used for the assay.*


9. Use the **Focus** controls (*Coarse Focus* or *Fine Focus*) by tapping gently on individual controls to adjust the focus of cells in the viewing pane until proper focus is achieved. Good focus is key for obtaining accurate cell counts. Cells in focus will have a bright center and a dark, crisp clearly defined edge as shown in the example below.
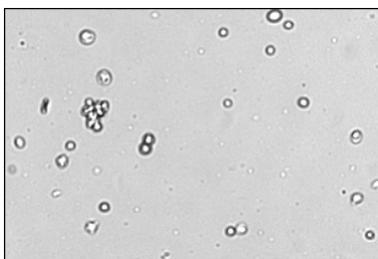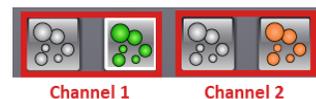
10. If the current assay uses fluorescent imaging, tap the *Green* and/or *Red* image icon. Confirm fluorescent spots have sharp edges. If the edges appear to be fuzzy, adjust focus and fluorescent exposure (using the **Exposure** controls) as necessary. If a second fluorescent image is available, adjust FL Exposure Time as necessary.

   Note: *If both Green and Red fluorescent images are available, a slight offset exists between the Brightfield 1 and Brightfield 2 images due to the distance that the camera had to move between the two filter sets.*

11. Tap the **Count** icon located in the bottom panel of the screen. A progress bar indicates counting in progress. A numeric counter also displays updated cell counts.
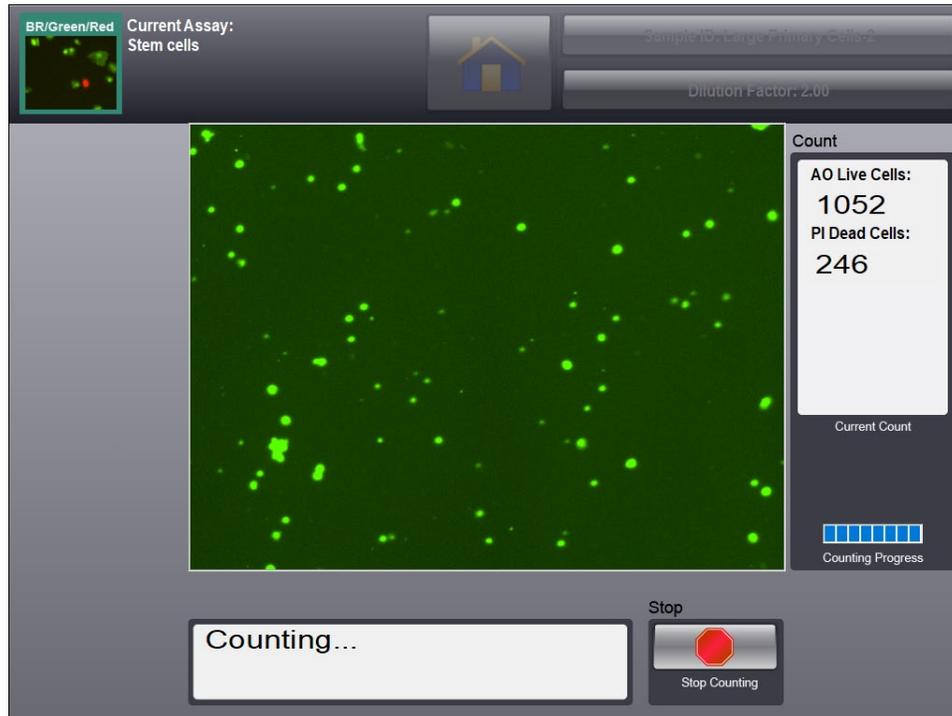
Count results are automatically displayed when the count is complete.

REVIEW COUNT RESULTS

1. Review the Count Results screen to confirm cells are being counted correctly. If the count results displayed appear to be correct, tap the **Details** icon to observe sample images or the **Next Sample** icon if you would like to count another sample (by repeating *Perform Cell Counting* steps on page *35*).
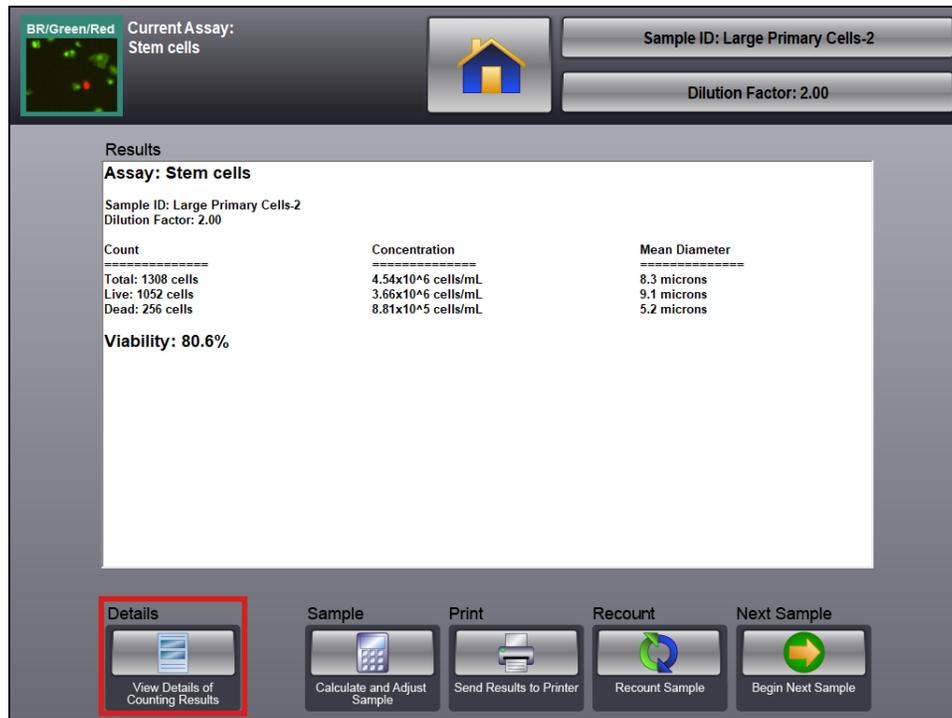
*The format used for presenting count results is defined by the assay Result Template. See Managing Templates on page *55 for details.*

2. After selecting the **Details** icon to observe cell images, tap the *Green* fluorescent image icon in the bottom panel, followed by the **View Counted Image** icon on the left of the screen to view counted cells. Enlarge the image by tapping the **Zoom In (+)** icon. Confirm that all of the live (green fluorescent) cells are circled in green.
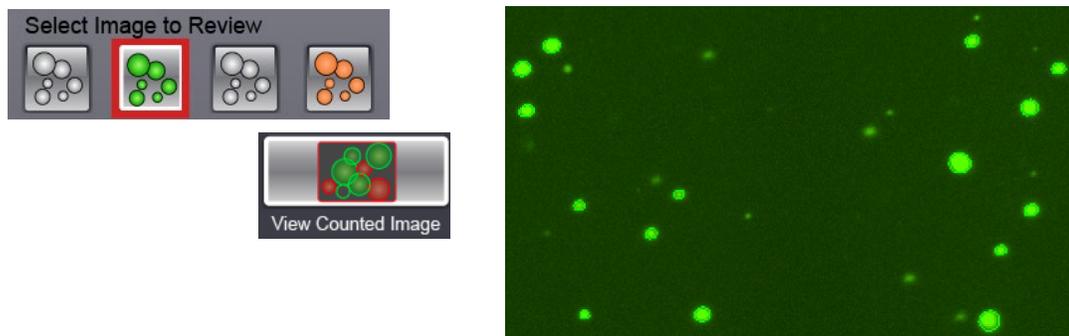
3. Tap the *Red* fluorescent image icon in the bottom panel, followed by the **View Counted Image** icon on the left of the screen to view counted cells. Enlarge the image by tapping the **Zoom In (+)** icon. Confirm that all of the dead (red fluorescent) cells are circled in green.
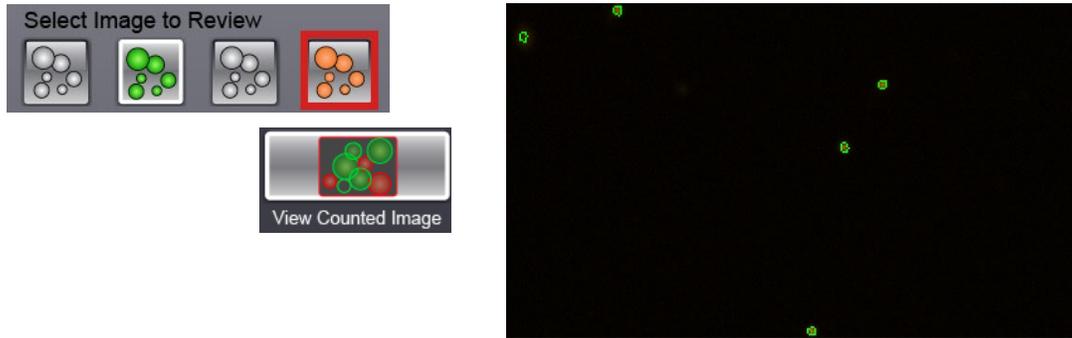
4. Tap the **View Combined Image** icon to view both the Red and Green flourescent images together. In addition, tap the **View Counted Image** icon to toggle display of the Counted graphic overlay.
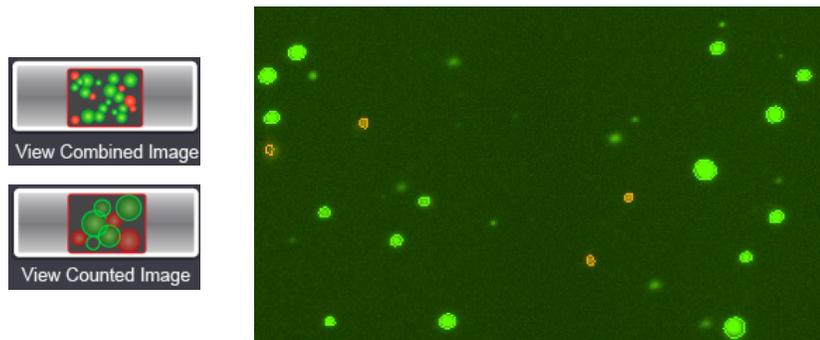
5. Tap the **Return** icon to return to the Count Results screen and review the *Cell Count*, *Concentration*, *Mean Diameter* and *Viability* percentage displayed.

Assay: Stem cells

Sample ID: Large Primary Cells-2
Dilution Factor: 2.00

Count	Concentration	Mean Diameter
=============	=============	=============
Total: 1308 cells	4.54x10^6 cells/mL	8.3 microns
Live: 1052 cells	3.66x10^6 cells/mL	9.1 microns
Dead: 256 cells	8.81x10^5 cells/mL	5.2 microns

Viability: 80.6%

Best Practices and Workflow Tips

- The progress bar at the bottom of the software panel indicates counting is in progress. A numeric counter also displays updated cell counts.

- Count results displayed represent the entire counting chamber and include *Cell Count*, *Mean Diameter (micron)* and *Cell Concentration (cells/mL)*.

- If a viability assay is selected, additional count results are displayed including live *and* dead cell counts (i.e., *Live/Dead Count*, *Total Cell Count*, *Live Cell Concentration*, *Total Cell Concentration*). *Viability* percentage is calculated with live cell count as a percentage of total cell count, including live and dead cells. *Viability testing can only be done with the addition of a Brightfield live/dead stain like trypan blue is added to the cell sample.*

- If cell counting optimization is required, you can customize cell type parameter settings associated with an assay. See *Editing Cell Types* on page *63* for details.

  Note: If you need help optimizing cell type parameter settings, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

- After modifying cell type parameters, tap the **Recount** icon 🔄 to re-analyze images for current scan and perform a new count.

- Data analysis features include *Sample Adjustment* and *Cell Size Analysis* tools. Based on the measured cell concentration, you can calculate the sample adjustment to achieve a target concentration or cell number. In addition, cell size data for the sample can be displayed using a histogram. See *View Cell Size Analysis* on page *40* and *Calculate Sample Adjustment* on page *45* for details.

Saving Data and Images

- To save count result data and images while viewing individual samples, tap the **Save Copy of Data** icon located on the Count Details screen. See *Saving Count Results* on page *25* for details. *All images captured for the sample during counting (i.e., A, B, C, D per channel in the current assay) will be saved as specified.*

- To save count result data and images automatically for all samples, enable the *Auto Save* instrument setting. See *Setting Up Auto Save Feature* on page *81* for details. *All images collected for the sample during counting (i.e., A, B, C, D per channel in the current assay) will be saved as specified.*

Printing Data

- Once a count has been performed, results can be printed by selecting the **Print** icon from the bottom panel of the Count Results screen. *The format used for presenting count results is defined by the assay Print Template. See Managing Templates* on page *55 for details.*

- To print count results automatically for all samples, enable the *Auto Print* instrument setting. See *Setting Up Auto Print Feature* on page *82* for details.

VIEW CELL SIZE ANALYSIS

The *Cell Size Analysis* tool displays cell size data from count results by generating a histogram that enables users to optimize cell diameter parameters for the sample (e.g., to exclude debris or very large cells) and refine count data. By overlaying data from multiple samples into a single histogram, users can perform an analysis of the change in cell diameter size over time.

To use the *Cell Size Analysis* tool, you must first perform a count on either a live or saved image to display the diameter for each object found, as well as the mean diameter for cells in your sample. Tap the **View Size Histogram** icon on the Count Details screen to plot this information into a *Cell Diameter Histogram* illustrating cell size distribution in the sample.

The histogram below shows cell size diameter distribution in relation to count results and contains *Green* and *Red* bars (i.e., representing both fluorescent channels). *The number of colored bars displayed will vary based on channels defined for the assay.* The *Cell Size Analysis* tool plots *Diameter Microns* along the *X* axis and total cell count (*Number of Cells*) along the *Y* axis by default. The scales for each axis are determined by count results.

1. **Count Results Data** – For each cell diameter, displays counted cells for all channels used in the assay.

2. **Histogram** – Displays a histogram (or chart) depicting count results data. *Data plotted along the X axis (i.e., Diameter Microns) and Y axis (i.e., Number of Cells) represent default settings and can be customized.*

3. **Chart Data Buttons** – To display the full data table created by the *Cell Size Analysis* tool as well as various other charts showcasing key aspects of the data (e.g., *F1 Intensity*, *F2 Intensity*, *F1 and F2 Intensity* and *F1 vs F2 Intensity*, tap any of the buttons displayed in the left corner of the bottom panel. See *Displaying Chart Data* on page *41* for a description of the function of each button.

4   **Chart Action Buttons** – Enables users to perform various actions to save, copy, print or further refine/organize details of the histogram. *Chart Action buttons may be disabled based on the nature of data being displayed using the Chart Data buttons.*

Save – Saves cell size diameter data for the sample to a file.

Load – Allows users to add multiple cell size diameter data files to the displayed chart by overlaying a graphical line (using different colors) for each file. *Data files are loaded one at a time using this button.*

Copy – Copies chart (as currently displayed) to the Windows clipboard for use in other applications.

Gates – Allows users to sort count results into meaningful subsets of the data by establishing gates based on minimum/maximum cell size diameters. See *Establishing Cell Diameter Gates* on page *43*.

Customize – Allows users to modify and refine chart parameters to meet their specific business needs. See *Customizing Chart Parameters* on page *44*.

Print Chart – Sends the histogram (as currently displayed) to a printer.

Viewing Chart Data

The following *Viewing Chart Data* buttons are available in the bottom left corner of the screen and allow users to display different aspects of count results data.

DATA TABLE

Tapping the **Data Table** button displays the full data table generated by the *Cell Size Analysis* tool.

| Obj Num	Image	X Pixel Pos	Y Pixel Pos	Obj Type	Diameter (um)	F1 Intensity (counts)	F2 Intensity (counts)
1	Image A	350	10	Cell F1 Positive	5.41	15026.0	0.0
2	Image A	1153	15	Cell F1 Positive	8.60	8552.0	0.0
3	Image A	381	16	Cell F1 Positive	5.56	13895.0	0.0
4	Image A	112	19	Cell F1 Positive	7.76	8178.0	0.0
5	Image A	269	22	Cell F1 Positive	6.42	5863.0	0.0
6	Image A	670	22	Cell F1 Positive	8.99	10388.0	0.0
7	Image A	1243	26	Cell F1 Positive	8.60	9961.0	0.0
8	Image A	625	34	Cell F1 Positive	4.91	3450.0	0.0
9	Image A	705	35	Cell F1 Positive	8.08	8819.0	0.0
10	Image A	980	42	Cell F1 Positive	8.50	9746.0	0.0
11	Image A	514	44	Cell F1 Positive	10.24	14167.0	0.0
12	Image A	785	49	Cell F1 Positive	12.23	18262.0	0.0
13	Image A	650	52	Cell F1 Positive	8.70	9994.0	0.0
14	Image A	994	59	Cell F1 Positive	7.65	7695.0	0.0
15	Image A	203	66	Cell F1 Positive	13.63	23943.0	0.0
16	Image A	707	66	Cell F1 Positive	9.81	11476.0	0.0
17	Image A	1051	66	Cell F1 Positive	10.57	14592.0	0.0
18	Image A	113	66	Cell F1 Positive	8.99	11425.0	0.0
19	Image A	335	66	Cell F1 Positive	8.70	10572.0	0.0
20	Image A	1099	66	Cell F1 Positive	7.98	8316.0	0.0
21	Image A	72	68	Cell F1 Positive	4.35	3284.0	0.0
22	Image A	832	70	Cell F1 Positive	6.15	5202.0	0.0
23	Image A	516	75	Cell F1 Positive	7.76	8344.0	0.0
24	Image A	835	77	Cell F1 Positive	8.80	10140.0	0.0
25	Image A	1024	85	Cell F1 Positive	15.95	30258.0	0.0

To save this table for future use (e.g., to be loaded into a future histogram as a record of cell size diameter data captured at a point in time, tap **Save** to save this table to a file.

FLUORESCENT INTENSITY

Tapping the **F1 Intensity** or **F2 Intensity** buttons displays a histogram plotting *Cell Intensity (Counts)* against *Number of FL Positive Cells* using only data for the selected channel (*F1* or *F2*). *The Sample ID and time/date stamp are included at the top of the chart.*
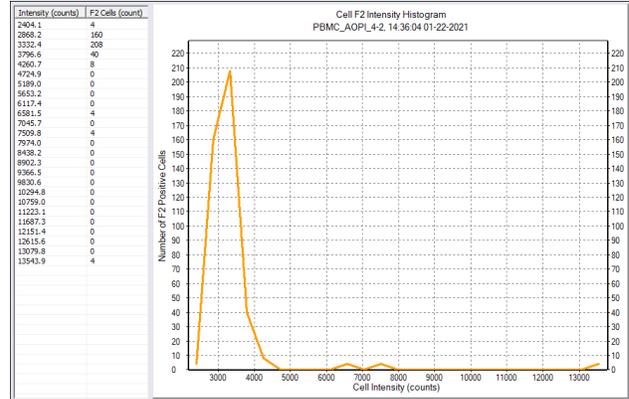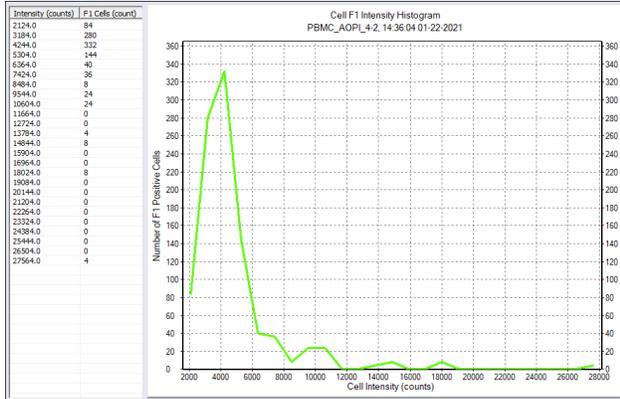
Tapping the **F1 Intensity**, **F2 Intensity** button displays a combined histogram plotting *Cell Intensity (Counts)* against *Number of F1,F2 Positive Cells*, overlaying data for both channels to optimize the comparison. Each graphical line is coded using the color representing the channel it represents – *Green* or *Red*. *The Sample ID and time/date stamp are included at the top of the chart.*
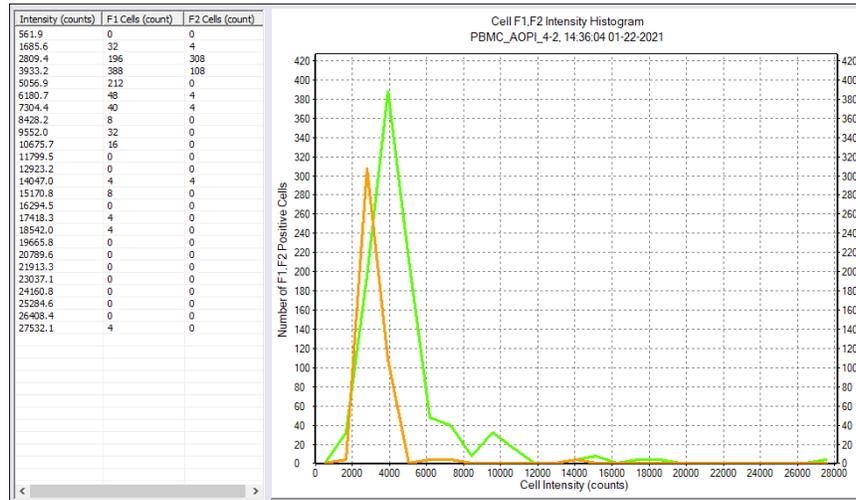
In comparison, tapping the **F1 vs F2 Intensity** button displays only those fluorescent positive cells that are common for both channels, plotting *F1 Intensity (Counts)* against *F2 Intensity (Counts)*. Dual Stain imaging mode is required to generate *Cell F1 vs. F2 Intensity* scatter plots.
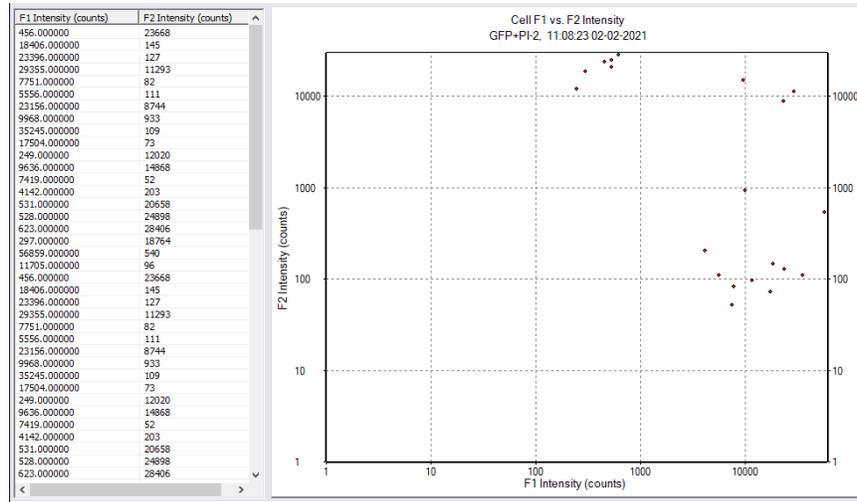

Establishing Cell Diameter Gates

Count results can be sorted into meaningful subsets of data by establishing 'gates' based on specified minimum size/maximum size cell diameters. Identifying the different cell populations that exist in your sample can assist with interpreting the results.

Tap the **Gates** button to display cell sample count results identifying the lower/upper limits of the *Count Diameter Range* of your sample, including a breakdown of *Concentration*, *Number of Counted Cells* and *Mean Cell Diameter* captured by each channel in the assay.

To add a new gate, tap the **Min Size** button, enter a value within the displayed *Count Diameter Range* representing the lower limit needed to identify count results for a specific cell type and tap **Done**. Repeat this process to enter a **Max Size** value for the upper limit needed to identify the cell type followed by **Done**, and then tap the **New Gate** button. Gate details are added to the panel including *Concentration*, *Number of Counted Cells* and *Mean Cell Diameter* captured for all cells in the specified *Gate Diameter Range* by each channel in the assay.

Continue to add new gates as necessary for individual populations in your cell sample. Click the **Clear Gates** button to clear all gates or the **Print Gates** button to print the specifications for all gates as they are displayed within the panel.

Customizing Chart Parameters

Users can modify and refine chart parameters to meet their specific business needs. For example, elements that can be customized include the width of histogram bars, the maximum scale value for the *Y* axis, the number of bins (i.e., groupings of data represented by bars) to be displayed, and the minimum/maximum cell diameter of counted cells to be presented (identifying the scale to be used for the *X* axis).

1. **Use Custom Histogram Bar** – Toggle control to *ON* position and enter widths for indicated bar types.

2. **Use Custom Scale** – Toggle control to *ON* position and enter the maximum number of cells to be displayed on the *Y* axis scale. *Indicate whether the Cell Size Analysis tool should always override the calculated scale (determined based on count results) with the entered maximum value when displaying histograms for this assay.*

3. **Use Custom Histogram Number of Bins** – Toggle control to *ON* position and enter the number of bins (i.e., groupings of data represented by bars displayed along the *X* axis) to be used when calculating histogram divisions. Bar widths are adjusted automatically to accommodate the specified number of bins. *Values can range from 5-50 bins.*

4. **Use Custom Size Range** – Toggle control to *ON* position and enter a custom cell diameter range (including both minimum and maximum values) for counted cells to be defined along the X-axis. *Indicate whether the Cell Size Analysis tool should always override the calculated scale (determined based on count results) with the values entered for this custom cell diameter range when displaying histograms for this assay.*

5. **Save Config** – Saves the customized configuration to a file (e.g., *MyChartSettings.ChartSettings*).

6. **Load Config** – Loads a configuration file from an external source.

7. **Save** – Saves customized parameters when using the *Cell Size Analysis* tool for the current assay.

CALCULATE SAMPLE ADJUSTMENT

The *Sample Adjustment* tool assists you in calculating the adjustment necessary in order to achieve a specified target concentration or target number of cell*s* in a tube. Before calculating this adjustment, you must first perform a count on either a live image or saved image to display the current cell count and concentration (or live/dead count, total cell count, viability %, live cell concentration and total cell concentration if **Test Viability** was selected).

To calculate sample adjustment:

1. Tap on the **Sample** icon located at the bottom of the Count Results screen.

2. Indicate *Sample Adjustment Source* by choosing from available options. *Sources displayed will vary based on the current assay.*

3. Enter **Original Sample Volume** by tapping the input field to edit displayed value.

4. Indicate whether **Target** concentration value to be entered will refer to a *Number of Cells* or *Concentration*.

5. Enter **Target** concentration by tapping the input field to edit displayed value.

6. View calculation results for *Measured Sample* Concentration and *Total Cell Number in Sample*.

7. Perform *Sample Adjustment* instructions to achieve the cell sample goal.

REFERENCE OF CELL TYPE/BEAD SAMPLE IMAGES

Common cell type and bead sample images taken by the Auto 2000 are presented for your reference below.

Cell Type Sample Images

Jurkat Cells
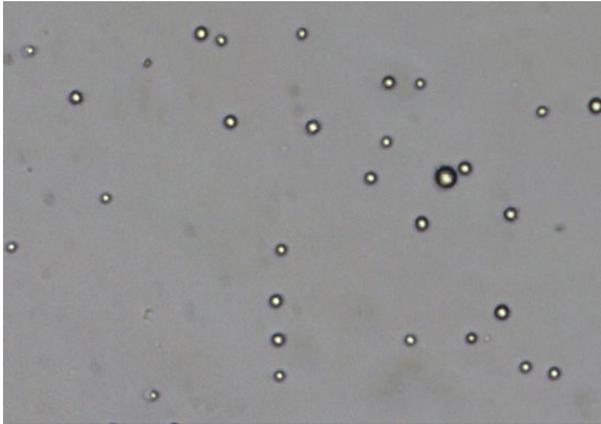
CD4 cells stained with Trypan Blue
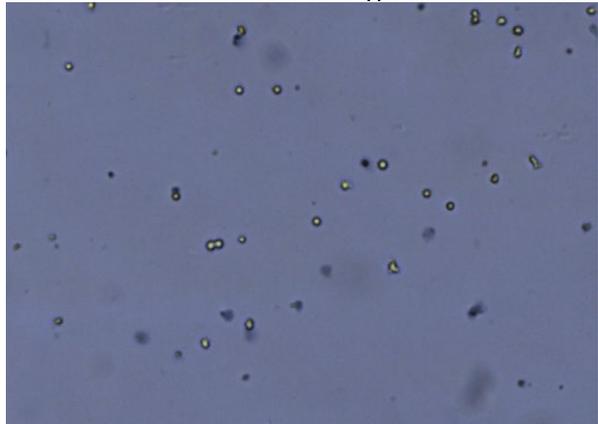
Bead Sample Images

5 µm Beads

10 µm Beads
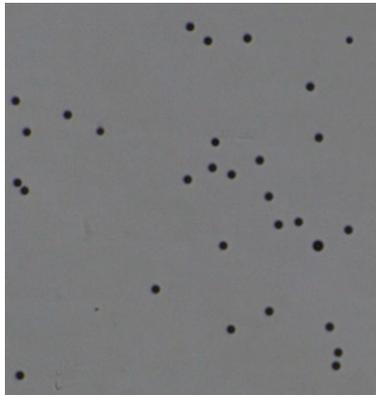
15 µm Beads
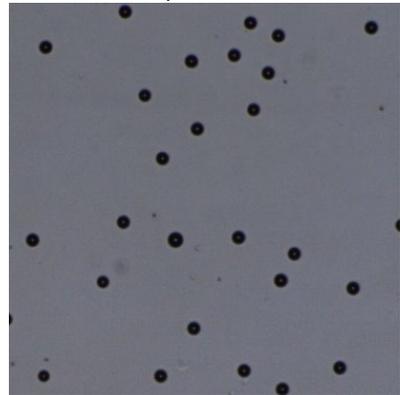
Chapter 10. Assays

This chapter describes customizable assays that can be modified to assist with cell counting and analysis.

The currently selected assay is highlighted in the *Assays Available for Selection* list with a red outline and is also displayed as the *Current Assay* in the top left corner of the screen.
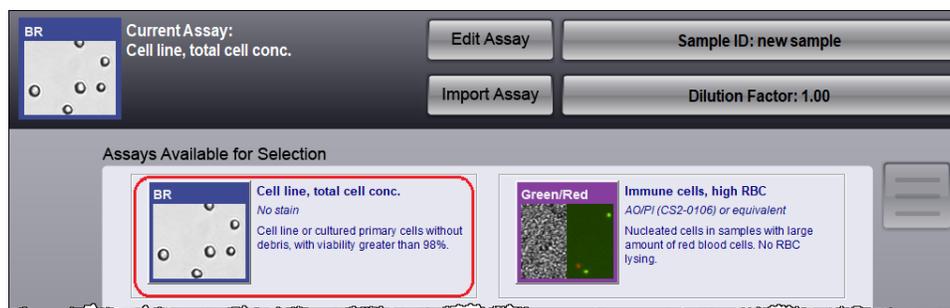
DEFAULT ASSAYS

The Auto 2000 software provides several assays that are displayed by default in the *Assays Available for Selection* list displayed on the Home screen.
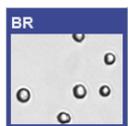
Cell Line, Total Cell Concentration
No Stain
Cell line or primary cells without debris, with viability greater than 98%.

After taking brightfield images of a sample, all cells are counted to determine total cell concentration.
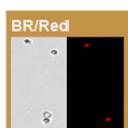
Cell Line, Viability Propidium Iodide
PI (CS1-0109)
Cell line or cultured primary cells without debris.

Propidium iodide (PI) is routinely used to determine cell viability. PI is a fluorescent stain that only penetrates dead cells and emits in the 'red' range (live cells are unaffected by PI).

After taking both brightfield and 'red' fluorescent images of a PI stained sample, all cells (from brightfield channel) and dead cells (from 'red' fluorescent channel) are counted to determine total and dead cell concentrations. Counts are then used to calculate percent viability.
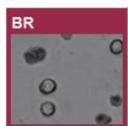
Cell Line, Viability Trypan Blue
Trypan Blue
Cell line or cultured primary cells without debris.

Trypan blue is routinely used to determine cell viability. Trypan blue penetrates and stains dead cells while leaving live cells unstained.

After taking brightfield images of a trypan blue stained sample, live and dead cells are counted to determine total, live and dead cell concentrations. Counts are then used to calculate percent viability.
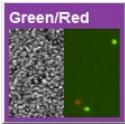
Immune Cells, High RBC
AO/PI (CS2-0160) or equivalent
Nucleated cells in samples with large amount of red blood cells. No RBC lysing.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images of an AO/PI stained sample, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

Immune Cells, Low RBC
AO/PI (CS2-0106) or equivalent
Nucleated immune cells after isolation in samples with some red blood cells. PBMC after ficoll separation, splenocyte without lysing RBC.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.
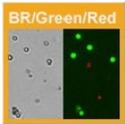
Primary Cells, Cell Lines
AO/PI (CS2-0106) or equivalent
Primary cells, cell lines or cell sample from dissociated tissue with debris.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

Stem Cells
AO/PI (CS2-0106) or equivalent
Stem cell sample.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

Users can edit parameter setting for assays displayed in this list and save them with a new name or import additional assays as necessary from either the built-in Assay Library installed with the software or an external network location. See *Importing Assays* on page *28* for details.

EDITING ASSAY PARAMETERS

Follow these steps to modify parameters for an assay or to create a new assay.

Editing Assay Name/Description

1. On the Home screen, select an assay in the *Assays Available for Selection* list and tap **Edit Assay.**
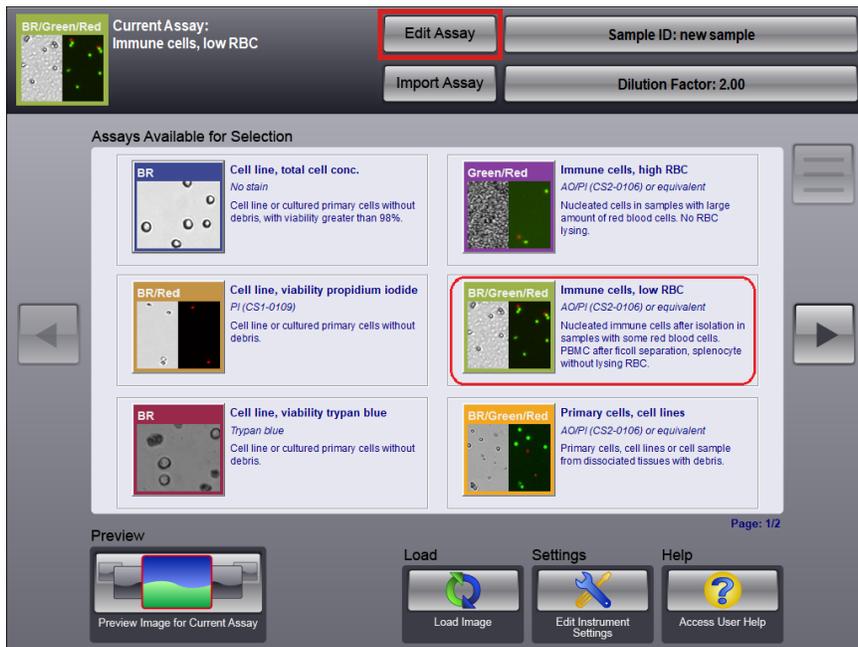
2. In the top section of the Assay Parameters screen, tap **Edit.**
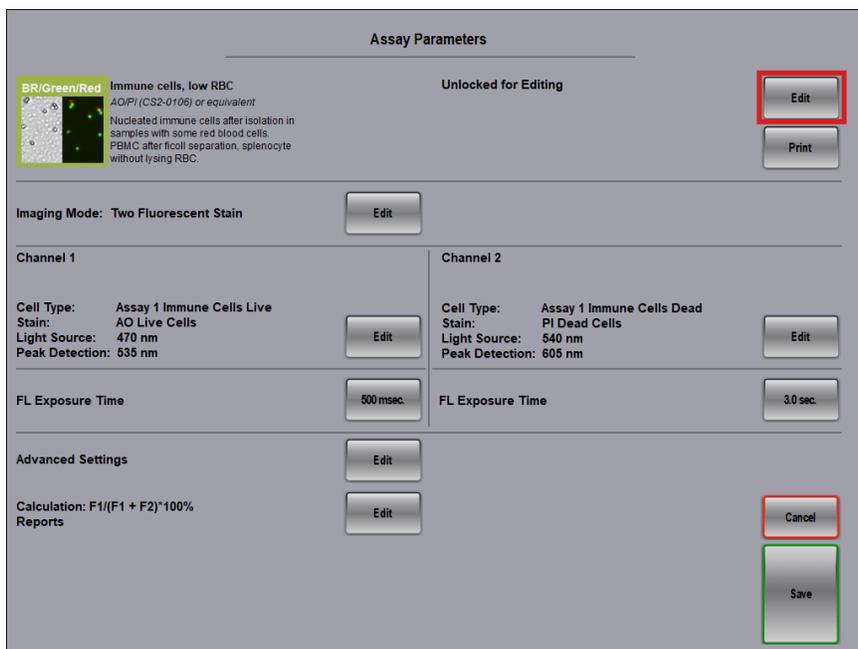
3. On the Assay Descriptions screen, enter a new **Assay Name** using the virtual keyboard that appears when you tap on the field. Tap **Done** when you are finished to close the keyboard.
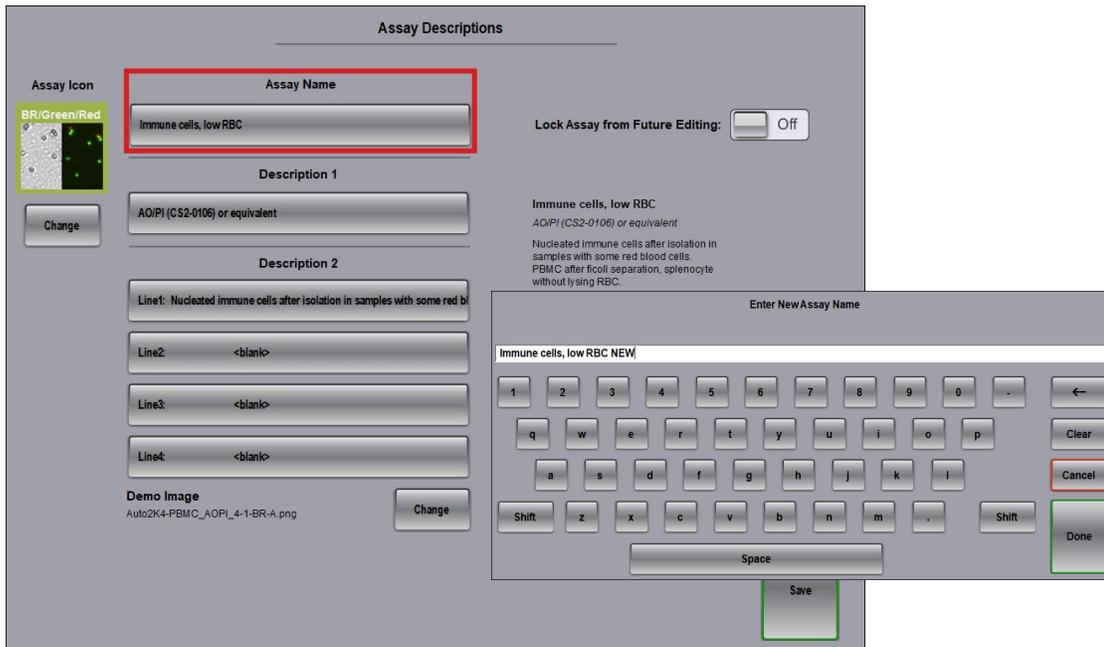

Note: Upon closing, you will be prompted to *Change the cell type name to match the new assay name or keep the old cell type name*. The cell type name is displayed in the *Channel 1* and *Channel 2* areas on the Assay Parameters screen. See *Editing Cell Types* on page *63* for details about editing cell types.

4. Modify details for **Description 1** and/or **Description 2** (*Lines 1-4*) as appropriate for the assay.

5. To change the image displayed as the *Assay Icon* in the upper left corner of the screen, tap the associated **Change** button to navigate and select a new assay icon image.

6. To change the *Demo Image* identified in the bottom left corner of the screen, tap the associated **Change** button to navigate and select a new demo mode image file set.

7. Indicate whether to **Lock Assay from Future Editing** by toggling the control to the *ON* position and click **Lock** in response to the confirmation prompt.

8. Tap **Save** to save description changes and return to the Assay Parameters screen.

Changing Assay Imaging Mode

1. To select an Imaging Mode for the assay that is different from the mode currently displayed (e.g., *Two Fluorescent Stain* in the screen below), tap **Edit** in the *Imaging Mode* area.

2. Select a new Imaging Mode for the assay and respond to confirmation messages as necessary.
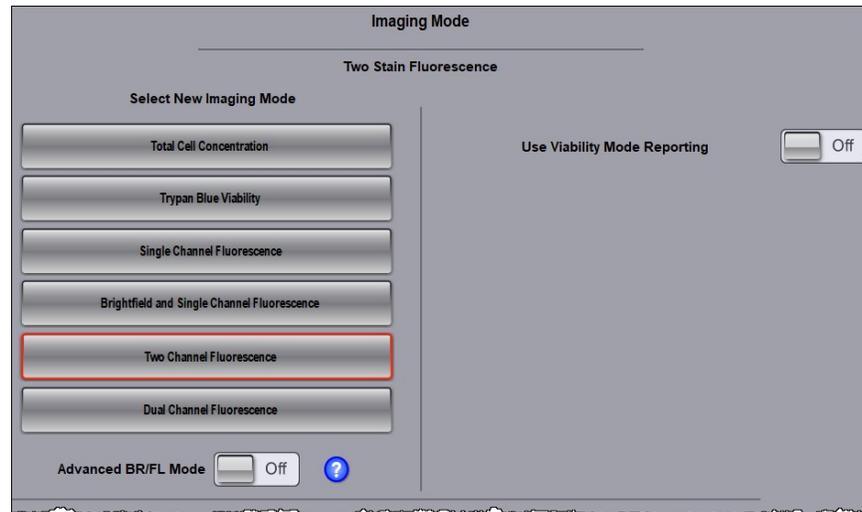
Available imaging modes are described below:

- **Total Cell Concentration** – Uses brightfield imaging to count cells and measure cell size.

- **Trypan Blue Viability** – Uses brightfield imaging in combination with trypan blue staining to count cells, measure cell size and determine cell viability.

- **Single Channel Fluorescence** – Uses one fluorescent channel (FL1) to count cells. *Although a brightfield image (BR1) is also displayed to assist with focus, it will not be counted.*

- **Brightfield and Single Channel Fluorescence** – Uses brightfield (BR1) imaging in combination with one fluorescent channel (FL1) to count cells.

- **Two Channel Fluorescence** – Uses two fluorescent channels (FL1 and FL2) to count cells. *Although brightfield images (BR1 and BR2) are also displayed to assist with focus, they will not be counted.*

- **Dual Channel Fluorescence** – Identifies cells that show fluorescence in *both* channels (FL1 and FL2).

Note: When changing the Imaging Mode for an assay, you will be prompted with a message indicating the action will invalidate the *DataTxt*, *Result* and *Print* templates currently assigned. Choose to **Keep** current templates, use **Default** templates or **Cancel** the action.

In addition, upon confirming you want to change the imaging mode, you may be presented with the Calculations and Reports screen to select a viability label and formula for the assay. See *Defining Assay Viability Calculation* on page *54* for details.

3. Indicate whether to use **Advanced BR/FL Mode** by toggling the control to the *ON* position. *This mode measures fluorescent intensity within all cells located in the brightfield image and can be used for a more accurate fluorescent count of clumped cells.*

4. Indicate whether to **Use Viability Mode Reporting** by toggling the control to the *ON* position.

5. Click **Save** imaging mode changes and return to the Assay Parameters screen.

Additional fields on the bottom of the Imaging Mode screen allow you to **Change** currently assigned custom templates (used for saving data, displaying count results and printing reports), revert to **Default** templates, edit an existing template or create a new template. See *Managing Templates* on page *55* for details.

Selecting Cell Type and Identifying Stain

1. To select a cell type for each channel in your assay that is different from the cell type currently displayed (e.g., *Assay 1 Immune Cells Live* for both channels in the screen below), tap **Edit** in the *Channel 1* or *Channel 2* areas.
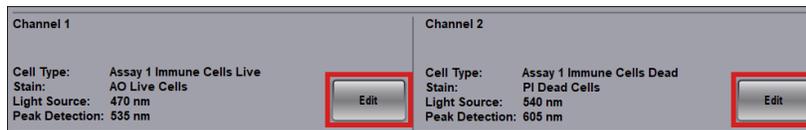

2. In the Channel 1/Channel 2 Settings screens, tap **Select New** to display available cell types.
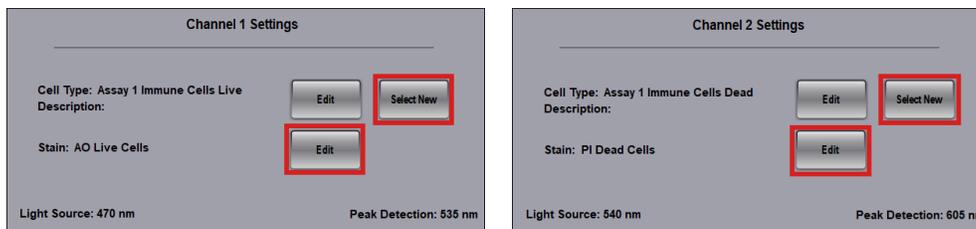
3. Select a cell type from the *Cell Types in Drop-down Menu* pane (displayed on the right) and tap **Done**.
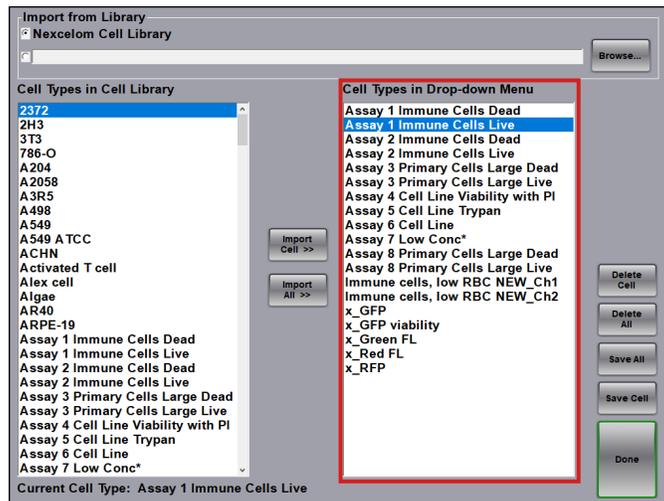

Note: If the cell type needed is not displayed in the *Cell Types in Drop-down Menu* pane, you can either import it from the built-in Cell Library by highlighting it in the *Cell Types in Cell Library Pane* (displayed on the left) and then tapping **Import Cell >>** to copy it to the *Cell Types in Drop-down Menu* pane or import it from a custom library. See *Importing Cell Types* on page *71* for details.

4. Confirm that the selected cell type is now displayed in the Channel 1/Channel 2 Settings screen as appropriate.

5. To identify the stain used in the cell sample, tap **Edit** and enter the name of the stain.

6. Tap **Save** to return to the Assay Parameters screen.

Editing Assay FL Exposure Time

To change fluorescent exposure time displayed for each channel in the assay (e.g., *500 msec.* for Channel 1 and *3.0 sec* for Channel 2 in the screen below), tap the current value for each channel and modify as necessary.
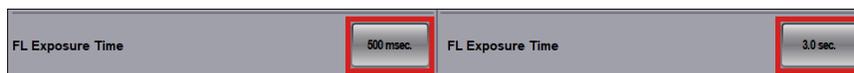
EDITING ADVANCED ASSAY SETTINGS

Tap the **Edit** button in the *Advanced Settings* area (bottom section) of the Assay Parameters screen to edit advanced assay parameters.
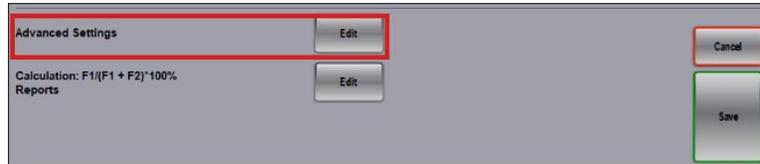
Advanced Settings can be used to vary the Brightfield exposure time factor, enable specialized counting algorithms for specific cell types, and set a default dilution factor.
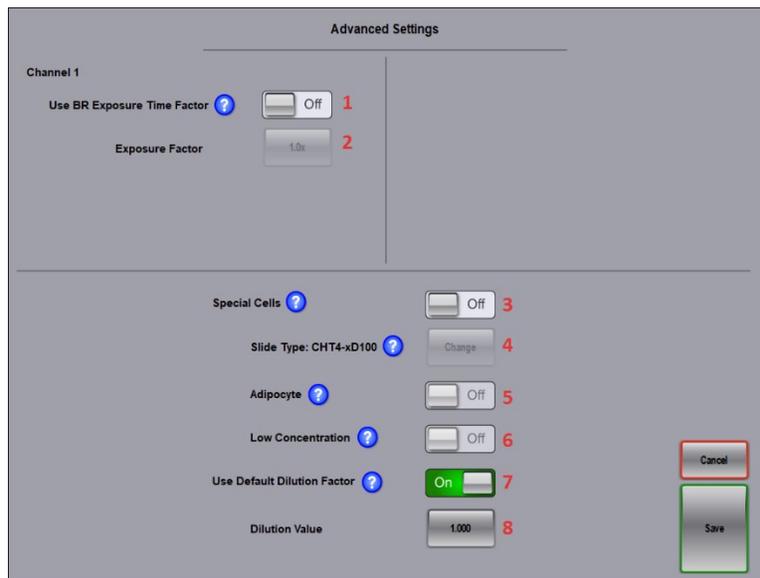

1. **Use BR Exposure Time Factor** – Changes the Brightfield exposure time by the displayed factor.

2. **Exposure Factor** – Displays the factor by which to change Brightfield exposure time. *Use BR Exposure Time Factor parameter must be selected for this input field to be enabled.*

3. **Special Cells** – Applies special counting algorithms and allows for change of counting chamber slide type. *If selected, enables the Slide Type, Adipocyte and Low Concentration parameters.*

4. **Slide Type** – Displays the current counting chamber slide type. *Special Cells parameter must be selected for this field to be enabled.*

5. **Adipocyte** – Applies special counting algorithms for adipocyte cells. *Special Cells parameter must be selected for this field to be enabled.*

6. **Low Concentration** – Enables counting of all four images for a cell sample as a single image. *Special Cells parameter must be selected for this field to be enabled.*

7. **Use Default Dilution Factor** – Enables users to set a default dilution factor for the assay.

8. **Dilution Value** – Displays the dilution factor set for the assay. *Use Default Dilution Factor parameter must be selected for this input field to be enabled.*

DEFINING ASSAY VIABILITY CALCULATION

Tap the **Edit** button in the *Calculation* area (bottom section) of the Assay Parameters screen to define the *Percent Viability* formula calculation for the current assay and select appropriate report templates.
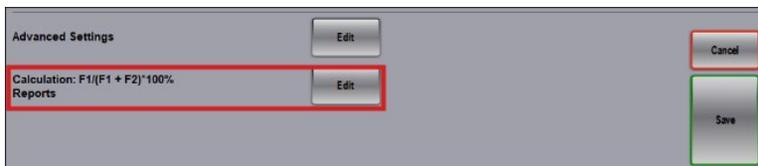
Indicate whether to **Use a Custom Formula Label** by toggling the control to the *ON* position, then tap the label button (e.g., *% Viability* in the screen below) to enter a new label using the virtual keyboard. Tap **Done**.
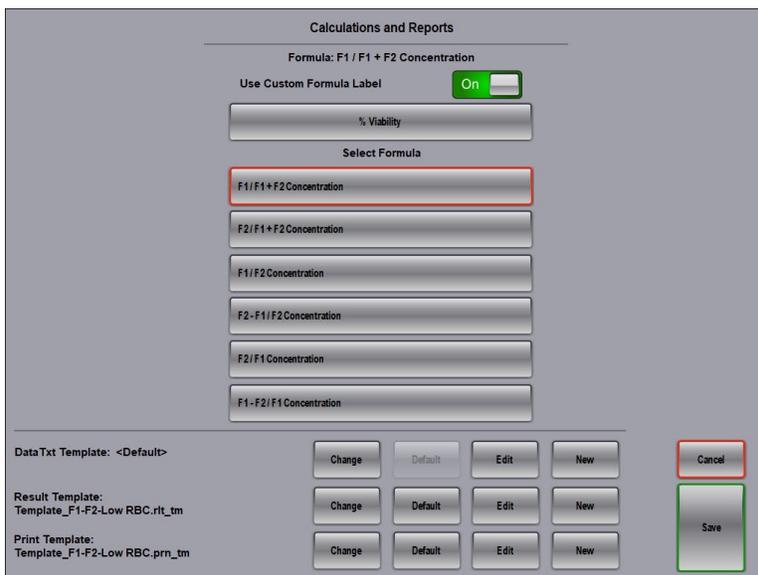
Select a *Percent Viability* formula calculation for the assay and respond to confirmation prompts as necessary. Tap the **Save** button to return to the Assay Parameters screen and then **Save** once again to return to the Home screen.

Additional fields on the bottom of the Calculations and Reports screen allow you to **Change** currently assigned custom templates (used for saving data, displaying count results and printing reports), revert to **Default** templates, edit an existing template or create a new template. See *Managing Templates* on page *55* for details.

MANAGING TEMPLATES

Templates are used to modify the format of how count results are saved to a file, displayed for viewing and printed as reports. The following types of templates are available for the Auto 2000:

- **DataTxt Template** – Defines the format of how data is saved to the *data.csv* file (i.e., default data text file). *DataTxt templates* have been designed for each imaging mode based on details specific to that mode and are assigned to assays as defaults. DataTxt templates are identified by the "*datatxt_tm*" file extension (e.g., the *<Default>* template defined for the *Two Channel Fluorescence* imaging mode is *Template_FL1FL2.datatxt_tm*).

- **Result Template** – Defines the format of how data is displayed on the Count Results screen. R*esult templates* are identified by the "*.rlt_tm*" file extension (e.g., *Template_F1-F2-Low RBC.rlt_tm* as shown below).

- **Print Template** – Defines the format of how data is printed in a report. *Print templates* are identified by the "*.prn_tm*" file extension (e.g., *Template_F1-F2-Low RBC.prn_tm* as shown below).


Notes regarding templates:

- Editing templates requires use of a physical keyboard and mouse. You will be prompted to confirm these devices are connected to the instrument via USB ports before you can use the template editor.

- By launching the *Template Editor* available from either the Imaging Mode screen or the Calculations and Reports screen, you can change currently assigned templates, revert to default templates, or edit and create new templates for each assay based on the needs of your organization.

- When editing a default DataTxt, Result or Print template, you will be prompted with a message indicating you are changing the default for *all* users and will be asked to confirm if you want to proceed.

- When changing the imaging mode for an assay, you will be prompted with a message indicating the currently selected Datatxt, Result and Print templates will be invalidated as details outlined in the templates will no longer accurately reflect data captured by the imaging mode. You can choose to **Keep** existing templates, revert to **Default** templates or **Cancel** the change request. *If you keep existing templates, another message will remind you to set correct Result and Print templates when editing of the assay is complete. Choosing to revert to default templates displays the Calculations and Reports screen where you can define a viability calculation and then review/manage templates. See Defining Assay Viability Calculation on page 54 for details.*

Editing Templates

1. In the *Imaging Mode* area of the Assay Parameters screen, select the **Edit** button.
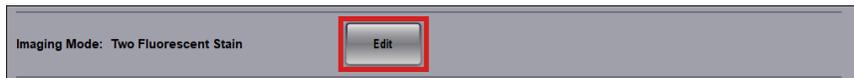
2. At the bottom of the Imaging Mode screen, tap the **Edit** button for the *DataTxt Template*, the *Result Template* file or the *Print Template* file to select the template you want to modify. *File extensions indicate template type (i.e., "datatxt_tm" for DataTxt Templates, ".rlt_tm" for Result Templates or ".prn_tm" for Print Templates).*


3. Connect a physical keyboard and mouse to the instrument via the USB ports and tap **Continue** in response to the confirmation prompt.

Note: If you choose to edit a default DataTxt, Result or Print template (i.e., display of the **Default** button for the template is disabled), you will be prompted with a message indicating that any changes made will affect *all* users of the instrument. Click **Continue** if you want to continue with changing the default template.

DATATXT TEMPLATES

When launching the template editor for a *DataTxt Template*, the template file currently assigned to the assay is displayed. There are three key areas in this template – the *Delimiter*, *Header Grid* and *Data Exporter Grid* sections.

Note: DataTxt templates have been designed for each imaging mode based on details specific to that mode and are assigned to assays as defaults. Although the *<Default>* indicator may be displayed on the Imaging Mode screen, the actual file name of the default file is displayed when editing the template (highlighted in screen below).
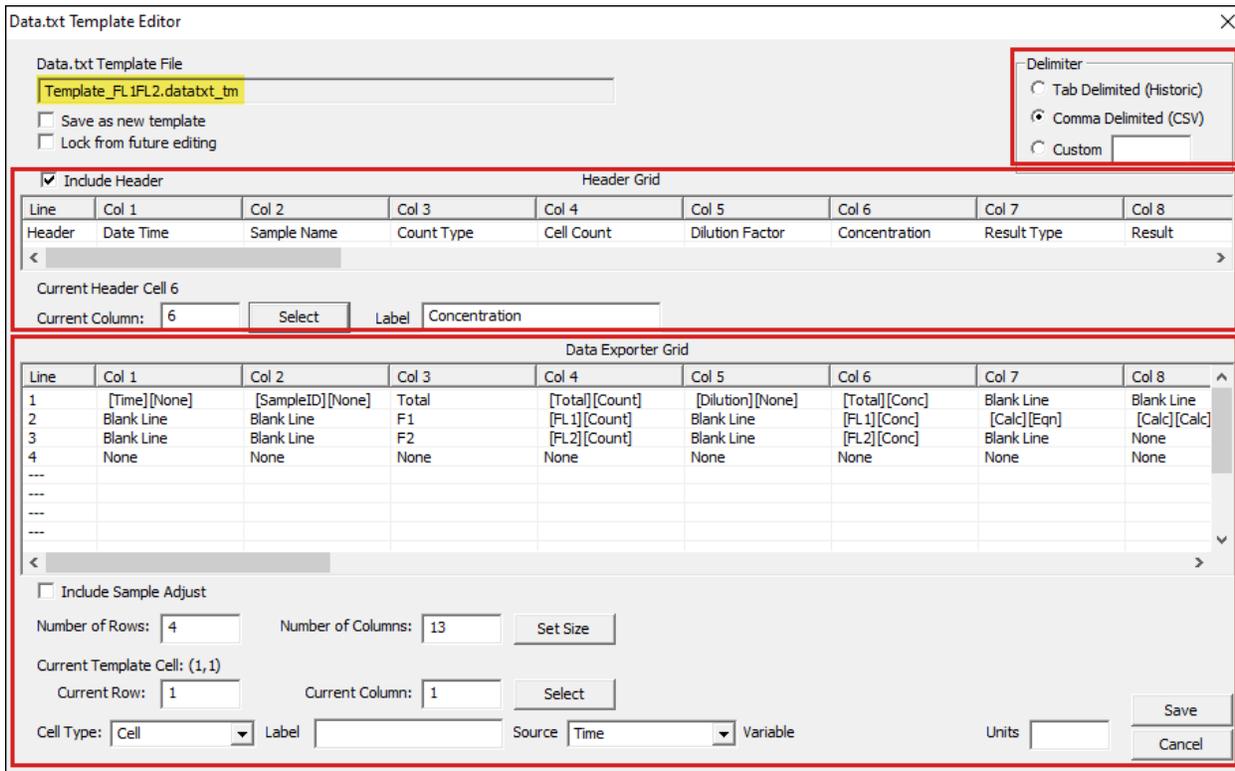
Select the **Save as new template** check box if you want to create a new template based on the selected template. The **Save** button at the bottom of the screen becomes **Save As** so you can enter a new file name when saving your changes to the file.

Select the **Lock from future editing** check box to prevent users from making changes to the template. *Once locked, the template can only be used as a source for creating new templates using the **Save as new template** check box.*

When editing of the DataTxt template is complete, verify its final format by performing a count on a sample using an assay to which the modified template has been assigned and then tapping the **View Data File** icon on the Count Details screen. The data file generated from the template shown above is presented in the screen below.

Changing File Delimiters

The type of delimiters defined within a DataTxt template determines the file extension used for data files generated as a result of using the template. The following options are available:

- *Tab Delimited* (i.e., plain text files that have historically been used by Auto 2000); generates *data.txt* files.

- *Comma Delimited* (i.e., plain text files consisting of comma-separated values that can be used with a spreadsheet application such as MS Excel or Google Sheets); generates *data.csv* files.

- *Custom* delimiters. Enter the character to be used as the delimiter; generates *data.txt* files.

Modifying the Header Grid

In the *Header Grid* section, use the horizontal scroll bar to view all column headings.


To modify a specific heading, enter the column number in the **Current Column** field, click the **Select** button to display the heading in the **Label** field and edit the label as necessary. *Changes made to column headings are added as you enter them and do not need to be saved until you complete all modifications.* Enter more column numbers to modify other headings or move on to another section in the template.

If you do *not* want column headings to be displayed in the template, clear the **Include Header** check box.

Modifying the Data Exporter Grid

In the *Data Exporter Grid* section, use the horizontal and vertical scroll bars to view the format contents of all columns and rows.


If you want to display sample adjustment calculations made for the sample at the bottom of the template, select the **Include Sample Adjust** check box.

To modify the structure of the template, change values displayed in the **Number or Rows** and **Number of Columns** fields, then click the **Set Size** button. If you *increase* the numbers of rows/columns, new lines are inserted below existing lines and new columns are added to the right of the last column. A default value of "None" is displayed in each cell added to the grid as a placeholder with a **Cell Type** set to *Blank*. If you *decrease* the number of rows/ columns, existing lines are deleted from the bottom and columns are removed from the right side of the grid.

To modify the *Format Contents* of any cell in the grid, follow these steps:

1. Specify the coordinates for the cell in the **Current Row** and **Current Column** fields (e.g., *Current Row = 1* and *Current Column = 4* as shown in screen shown above), then click the **Select** button.

2. Select an option from the **Cell Type** drop-down (i.e., *Cell* or *Cell Label*). *Fields displayed for editing format contents will vary depending on the cell type.* If **Cell Type** is set to *Cell*, the following fields are displayed:


If **Cell Type** is set to *Cell Label*, only the **Label** field is displayed.

3. To edit format contents of the selected cell, modify values in the **Label**, **Source**, **Variable** and **Units** fields (if applicable) accordingly. *Display of the **Variable** field and the values used to populate it may vary depending on the value selected for the **Source** field.*

4. When your changes to the template file are complete, click **Save** (or **Save As** if the **Save as new template check box** was selected to enter a new file name before saving) to return to the Imaging Mode screen.

5. Click **Save** until you return to the Home screen, select the **Preview** icon to view the image for current assay, select the **Count** icon to display count results and then the **View Data File** icon on the Count Details screen. Confirm the format of your modified DataTxt Template.

RESULT AND PRINT TEMPLATES

When launching the template editor for a *Result Template* or *Print Template*, the template file currently assigned to the assay is displayed.

To modify the structure of a template by deleting, inserting or appending lines, use the mouse to position the cursor on the desired *Line* and click the **Delete Line**, **Insert New Line** or **Append New Line** buttons. New lines are inserted *above* the current line while appended lines are inserted *below* it.

To modify the *Format Contents* of an individual line in the template, follow these steps:

1. Use the mouse to position the cursor on the desired *Line* (note that *Current Template Line* indicator is updated to reflect the selected line) and select an option from the **Line Type** drop-down (e.g., *Blank*, *Page Break*, *1-3 Columns*, *1-2 Images*, *1-3 Concentrations* and *Adjust Report*). Fields displayed for editing format contents will vary depending on the selected line type.

2. To edit format contents of the selected line, modify values in the **Label**, **Source**, **Variable**, **Units** and **Align** fields (if applicable) accordingly. *You can add up to two images per line, specifying the **Type** (File or Instrument) and alignment. Additional fields may be displayed based on selected image type.*

3. To indent the line from the left margin, select the **Indent** check box.

4. If the contents of a line should be displayed in a larger font (e.g., labels), select the **Large Font** check box.

5. When your changes to the template file are complete, click **Save** to return to the Imaging Mode screen.

6. Click **Save** until you return to the Home screen, select the **Preview** icon to view the image for current assay and select the **Count** icon to display count results. Confirm the format of your modified Result Template and/or select the **Print** icon in the bottom panel of the screen to confirm the format of your Print Template.

Creating New Templates

1. Return to the Imaging Mode screen and tap the **New** button for the type of template file you want to create.
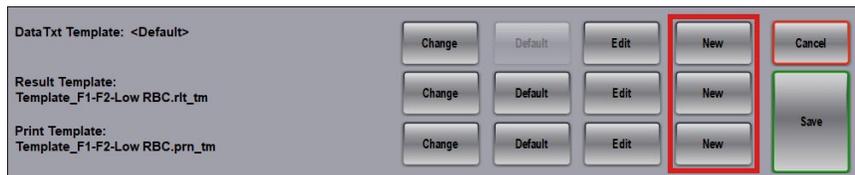
2. Connect a physical keyboard and mouse to the instrument via the USB ports and tap **Continue** in response to the confirmation prompt.

3. The template editor displays a new (blank) template file based on your selection (i.e., *DataTxt Template*, *Result Template* or *Print Template)*. Establish the format for a new template and click the **Save** button. See instructions for editing *DataTxt Templates* on page *57* or editing *Result and Print Templates* on page *60* for details regarding each template type.

4. Enter a **File Name** and save it to the default Auto 2000 *Template* folder by clicking the **Save** button.

    Note: If the newly created template should *not* be associated with the current assay, you will need to change the selected template using the **Change** button. *See details for changing selected templates below.*

Changing Assigned Templates

1. Return to the Imaging Mode screen and tap the **Change** button for the *DataTxt Template*, the *Result Template* file or the *Print Template* file to select the template you want to assign to the assay. *File extensions indicate template type (i.e., "datatxt_tm" for DataTxt Templates, ".rlt_tm" for Result Templates or ".prn_tm" for Print Templates)*. Note the name of the template file currently associated with the assay.

2. In the Select New screen, select a template file to be assigned to the assay by tapping on a file icon in the default Auto 2000 Template folder or navigate to an available drive and folder. *Only files with an extension for the template type selected (i.e., "datatxt_tm" for DataTxt Templates, ".rlt_tm" for Result Templates or ".prn_tm" for Print Templates) will be displayed in the Select file area of this screen.*

3. In the Imaging Mode screen, confirm that the name of the template file assigned to the assay has changed per your selection and click the **Save** button to return to the Assay Parameters screen.

Restoring Default Templates

To restore the *DataTxt Template, Result Template* or *Print Template* files to assay defaults, select the **Default** button at the bottom of the Imaging Mode screen for the template type.

Although the *<Default>* indicator is displayed on the Imaging Mode screen to identify that the default template file is being used, the actual file name of the default file can be found if you choose to edit the template.
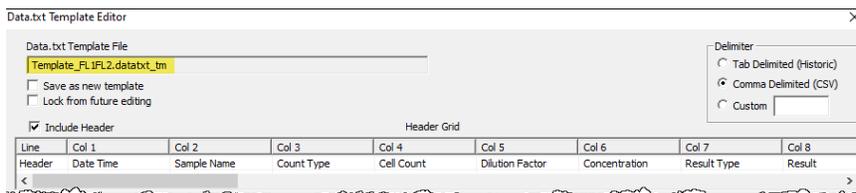
Note: If you choose to edit a default DataTxt, Result or Print template (i.e., display of the **Default** button for the template is disabled), you will be prompted with a message indicating that changes will affect *all* users. Select **Continue** if you want to continue with changing the default template.
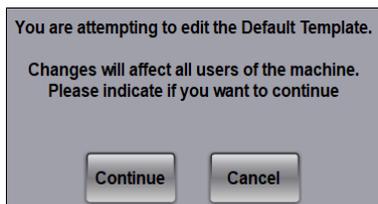
Chapter 11. Cell Types

This chapter describes editing parameters for cell types associated with assays and lists all cell types included in the built-in Cell Library installed with the software. In addition, it provides instructions for editing templates used for the display and printing of count results.

EDITING CELL TYPES

To edit the cell type for each channel in an assay, highlight the assay in the *Assays Available for Selection* list and tap the **Edit Assay** button, followed by tapping the **Edit** button in the *Channel 1* or *Channel 2* areas of the Assay Parameters screen.
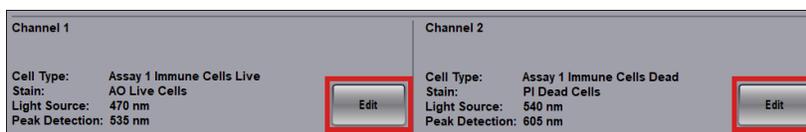
In the Channel 1/Channel 2 Settings screens, select the **Edit** button to display the Edit Cell Type Parameters screen.
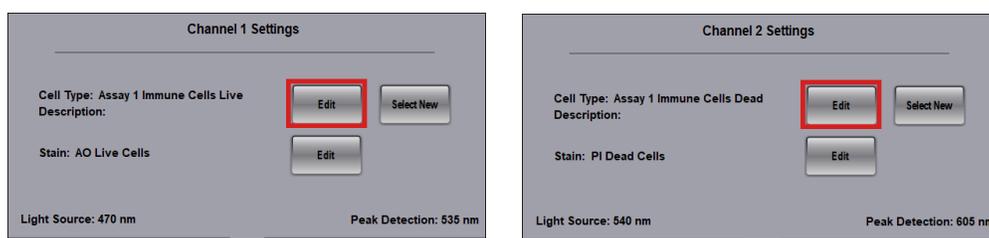
Note: To select a new cell type, tap the **Select New** button. See *Importing Cell Types* on page *71* for details.

Cell Type Parameters Screen

1   **Cell Type Name** – Identifies name given to the cell type. *Tap the **Edit** button to edit cell type name.*

2   **Description** – Contains detailed cell type description. *Tap the **Edit** button to edit cell type description.*

3   **Cell Diameter** – Indicates minimum (**Min Size**)/maximum (**Max Size**) cell diameters to be counted.

4   **Roundness** – Indicates minimum cell shape roundness factor to be counted. *Values range from 0.10 (includes all cell shapes) to 1.0 (includes only perfectly round cells).*

5   **Contrast Enhancement** – Defines contrast enhancement value for cells in relation to the background. *Values range from 0.01 (cells with high contrast to background) to 0.90 (cells with low contrast to background); recommended value is 0.4.*

6   **Decluster** – Defines whether individual cells within a clump are to be counted. *Turning this off allows clumps to be counted as one unit if its diameter falls within the min/max diameter range.*

7   **Edge Factor** – Indicates degree to which cell edges must be enhanced for optimal declustering. *Values range from 0.0 (clearly defined edges) to 1.0 (edges difficult to distinguish from background).*

8   **Threshold Factor** – Indicates the threshold ratio between cell signal and background. *Values range from 0.0 (cell signal to background is very low) to 1.0 (cell signal to background is high).*

9   **Background Adjust** – Indicates the adjust ratio between cell signal and background. *Values range from 0.0 (cell signal to background is very low) to 1.0 (cell signal to background is high).*

10 **Advanced Options, Trypan Button** – Defines trypan blue viability cell detection parameter settings.

11 **Advanced Option, FL Button** – Defines fluorescent viability cell detection parameter settings.

12 **Print Button** – Prints the cell type parameter settings.

13 **Cancel Button** – Cancels changes you have made to parameter settings and returns to previous screen.

14 **Save Button** – Saves changes to parameter settings.

ADJUSTING CELL DIAMETER PARAMETERS

For each cell type, the **Cell Diameter Min Size/Max Size** (in microns) parameter setting indicates the size of cells to be included in the count.

To determine if cell diameter parameters are set up properly, use the **View Counted Image** icon in the Count Details screen to display a graphical overlay outlining counted cells (e.g., green for counted cells and yellow for cells *not* counted as they are larger than the specified cell diameter). For example, the figure below shows counted cells for the same HT-29 image using different cell size parameter settings.
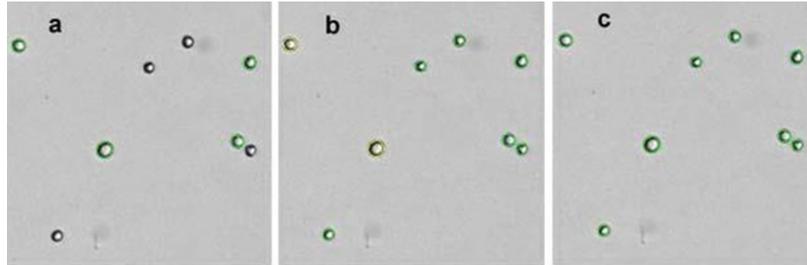
HT-29 cell count results were analyzed using different cell diameter parameter settings. Figure (a) – "Cell Diameter Min Size" size is too large. Cells smaller than 15 µm are not counted, as indicated by missing outlines. Figure (b) – "Cell Diameter Max Size" is too small. Cells larger than 16 µm are not counted, as indicated by the yellow outlines. Figure (c) – Both "Cell Diameter Min Size" and "Cell Diameter Max Size" are set up properly to include all cells.

Use the *Cell Size Analysis* tool to help you optimize cell diameter parameter settings for the sample by generating a histogram of cell size data from count results. See *View Cell Size Analysis* on page *40* for details.

ADJUSTING DECLUSTER PARAMETERS FOR CLUMPY CELLS

The **Decluster Edge Factor** and **Decluster Th Factor** (*Decluster Threshold Factor*) parameters may be used to separate clumpy cells (i.e., connected cells for which it is difficult to distinguish clearly defined edges). *Default values for these parameters are 0.5 and 1.0, respectively.*
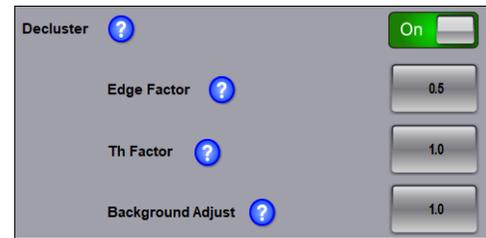
For most cell types, the default values for these parameters are adequate to separate clumpy cells. However, some cell types (such as MCF7 and PC12) contain cells having dim boundaries within the clump. In these cases, decluster parameters can be modified to visually separate clumpy cells in the captured images.

To define edges of clumpy cells in more detail, slowly increase the **Decluster Edge Factor** and **Decluster Th Factor** values. *These parameters work in conjunction with the **Contrast Enhancement** value on the Cell Type Parameters screen.*
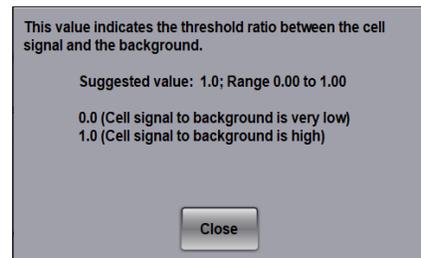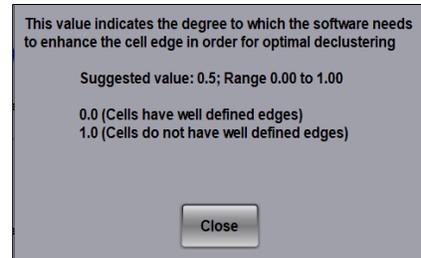
SAMPLE CLUMPY MCF7 CELLS

In the sample below, clumpy MCF7 cells are analyzed using both default and modified **Decluster Edge Factor** and **Decluster Th Factor** parameter settings.
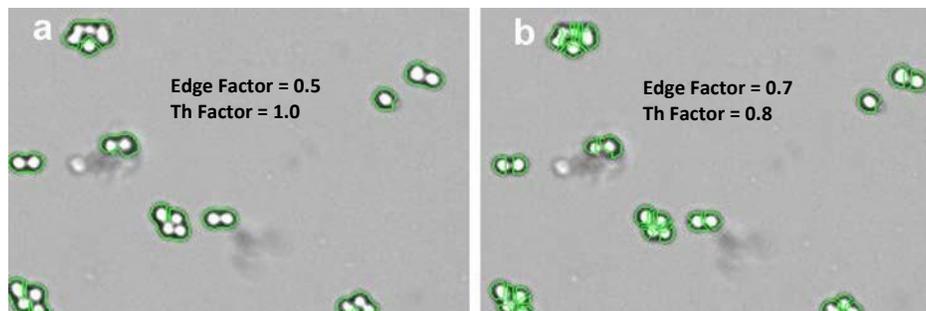
MCF7 cell count results were analyzed using different "Decluster" parameter settings. Figure (a) shows default decluster settings (cells within clumps are not fully separated), resulting in MCF7 concentration of 6.5×10^5 cell/mL. When modified declustering settings are used as shown in figure b, cells within the clumps are separated resulting in MCF7 concentration of 8.3×10^5 cell/mL.

DISABLING DECLUSTER FOR SMALL CELLS

Examples of small size cells are mouse splenocytes, lymphocytes, thymocytes, and PBMCs. Their cell diameters are typically less than 7 μm.

The Auto 2000's focus position for really small cells may influence cell counting when the *Decluster* feature is enabled (i.e., the **Decluster** parameter is *not* enabled). *This feature works in conjunction with the **Contrast Enhancement** value on the Cell Type Parameters screen.*
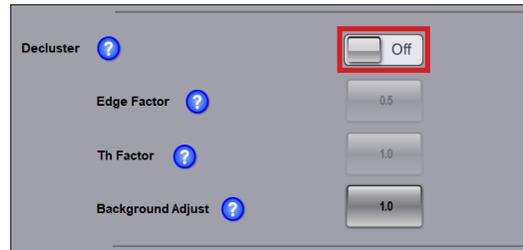
For the purpose of the following example, cell type parameter settings as shown in this screen were used to count mouse thymocyte cells in low concentration (i.e., cell counts less than 1300). Images explaining count results for various scenarios are presented.

Mouse thymocyte count results using variations in the **Decluster** parameter are shown below.
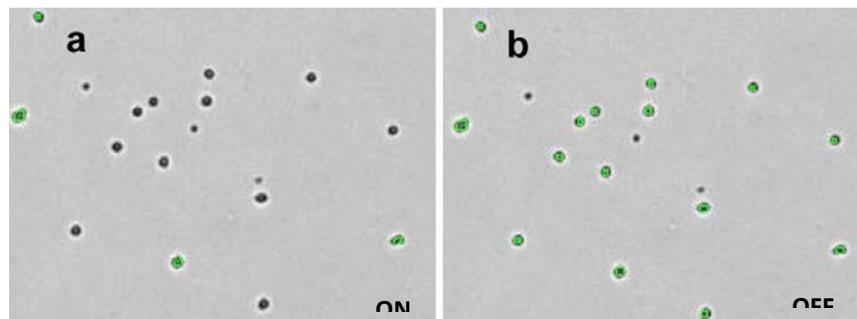
Mouse thymocyte count results are analyzed with Decluster parameter enabled in figure (a) and disabled in figure (b). The green outlines indicate counted cells. When Decluster parameter is not enabled, more cells are counted.

However, some samples contain clumpy cells, as shown in figure (a) below. Other samples have a very high cell concentration as shown in figure (b). For these samples, the *Decluster* feature is enabled and the **Contrast Enhancement** value was reduced (to a value of *.70*).
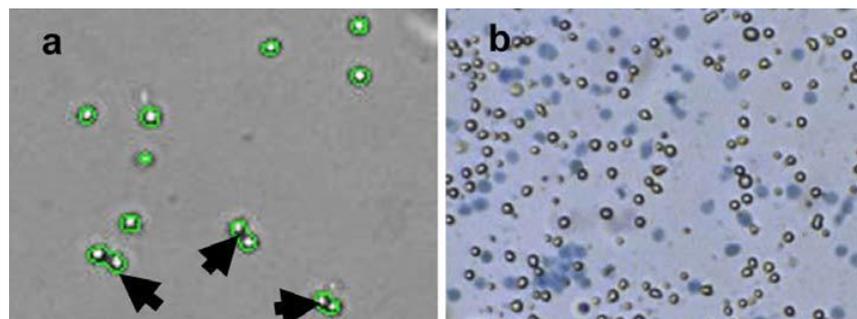
Cell type parameters for high concentration or clumpy small-sized cells.

ADJUSTING TRYPAN VIABILITY PARAMETERS

Using default settings for *Trypan* viability parameters provides the fastest counting speed for standard cell types stained a solid dark blue by trypan blue. As some cell types exhibit different morphology *after* being stained, it may be necessary to adjust parameters for accurate detection of the trypan blue population as indicated below. From the Cell Type Parameters screen, tap on the **Trypan** button in the *Advanced Options* area to display the Trypan Viability settings screen.
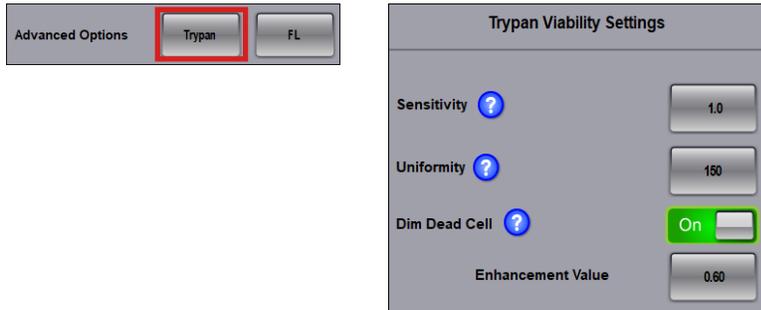
Sensitivity – Adjusts the darkness level of trypan blue stained cells to be included in dead cell count. *Values range from 0.10 (detects very dark stained cells to 5.00 (detects more mixed staining populations).*
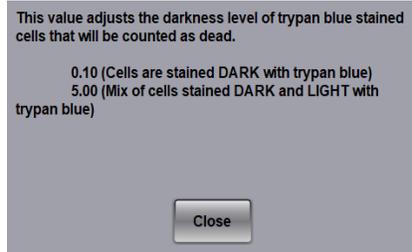
Uniformity – Indicates trypan blue staining uniformity to be included in dead cell count. *Values range from 100 (stained cells are all uniform in color) to 255 (stained cells have non-uniform dark and light areas).*
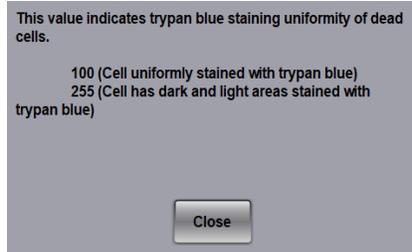
Dim Dead Cell – When feature is enabled (*ON*), helps to detect very dim stained dead cells.
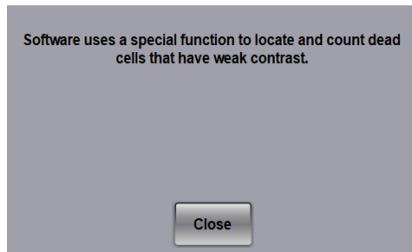
Enhancement Value – *Dim Dead Cell feature must be selected for this field to be enabled.* Refines the division between the background and cells with low contrast (i.e., no defined edges). *Suggested enhancement value is 0.60; values range from 0.04 (stained cells have medium contrast to background) to 0.90 (stained cells have very low contrast to background).*

WEAK BLUE DEAD CELLS

To pick up weak blue dead cells, enable the **Dim Dead Cell** feature and slowly increase the **Enhancement Value** to further refine the division between the background and cells with low contrast (i.e., no defined edges).

Note: It is a best practice to use the **Dim Dead Cell** feature in conjunction with other *Trypan* viability parameters (i.e., *Sensitivity*, *Uniformity or Enhancement Value*) to avoid overcounting.

DEAD CELLS WITH LIGHT BLUE CENTERS

To pick up dead cells with light blue centers, slowly increase the **Uniformity** value. *Suggested value is 150; available range is 100 (stained cells are all uniform in color) to 255 (stained cells have non-uniform dark and light areas).* In addition, slowly increase the **Sensitivity** value to further refine image clarity. *Suggested value is 1.0; available range is .10 (stained cells are all dark) to 5.0 (stained cells are a mix of dark and light images).*

Variations in sensitivity values used to pick up dead cells with light blue centers are presented in the figures below.
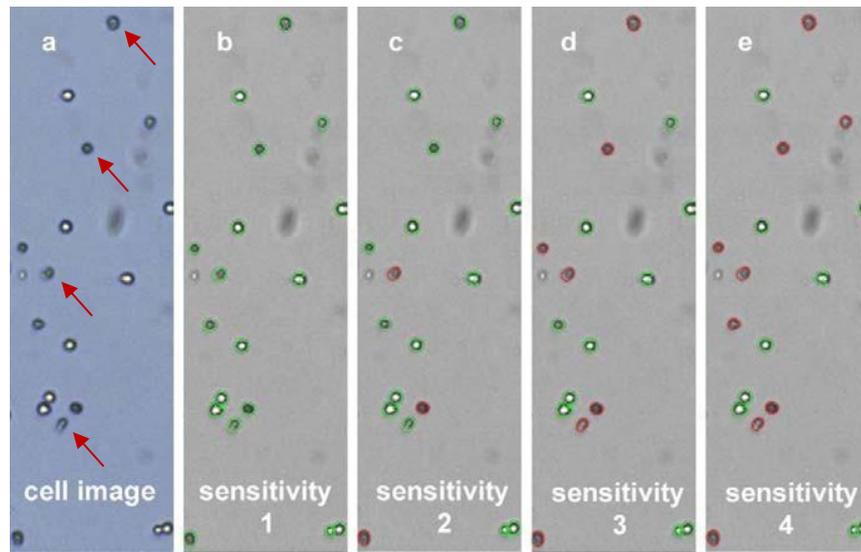
CHO cell count results were analyzed using different "Sensitivity" settings. Figure (a) shows CHO cell image while figures (b) – (e) show an incremental increase in "Sensitivity" parameter from 1 to 4, respectively. Green outlines indicate live cells while red outlines indicate dead cells. Both live/dead cell counts in figure (e) agree with visual observation of image in (a).

ADJUSTING FLUORESCENCE PARAMETERS

Using default *Fluorescence* parameter settings that have been defined for selected cell types provides the fastest counting speed for standard cell types stained appropriately for an assay. From the Cell Type Parameters screen, tap on the **FL** button in the *Advanced Options* area to display the Fluorosence settings screen.
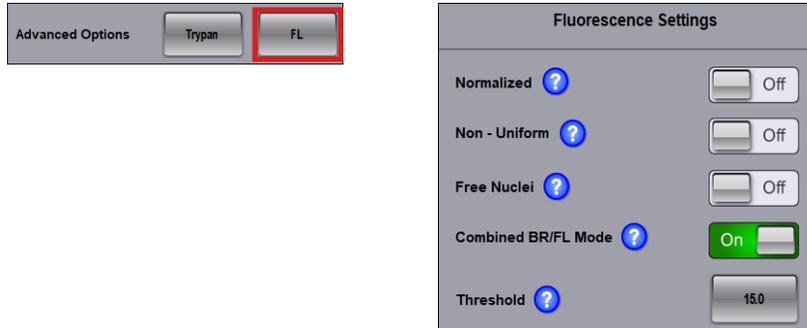

Normalized – When feature is enabled (*ON*), adjusts fluorescence intensity to the size of the cell. For example, if cells don't uptake the staining all at once and both light and dark areas exist within a cell, enabling this feature normalizes fluorescence intensity throughout the entire cell rather than having multiple intensity readings.

Non-Uniform – When feature is enabled (*ON*), identifies a cell as positive even if only a portion of the cell has fluorescence intensity above the set threshold. Enabling this feature is useful if cells in the sample have a mixed staining population that contains both light and dark areas.

Free Nuclei – When feature is enabled (*ON*), uses a proprietary image analysis algorithm that excludes free floating nuclei from being counted as cells.

Combined BR/FL Mode – When feature is enabled (*ON*), uses a proprietary counting method combining brightfield and fluorescence imaging to perform enhanced declustering.

Threshold – Indicates the percentage of fluorescence intensity required for cells to be counted as positive. As this feature establishes the maximum intensity of all images taken for the sample to be 100%, entering a threshold value representing a percentage of that maximum (e.g., 15.0 % as shown above) excludes cells with an intensity that falls below the set threshold from being counted. *Suggested threshold value is 5.0; values range from 3.0 (detects very dim cells) to 99.0 (detects only completely over-saturated cells).* As a general rule, lower the threshold value to include dimmer cells or increase the threshold value to exclude cells.

IMPORTING CELL TYPES

Cell Types define image analysis parameters used in cell sample detection. The *Import Cell Type Screen* is used to import either standard cell types from the built-in *Cell Library* installed with the software or custom cell types from an external network location.

Once a library is selected, you can highlight cell types from the library (displayed in the pane on the left) and import them to appear in the *Cell Types in Drop-down Menu* pane (displayed in the pane on the right).

To change the cell type selected for the current assay, highlight a cell in the *Cell Types in Drop-down Menu* pane and tap the **Done** button.
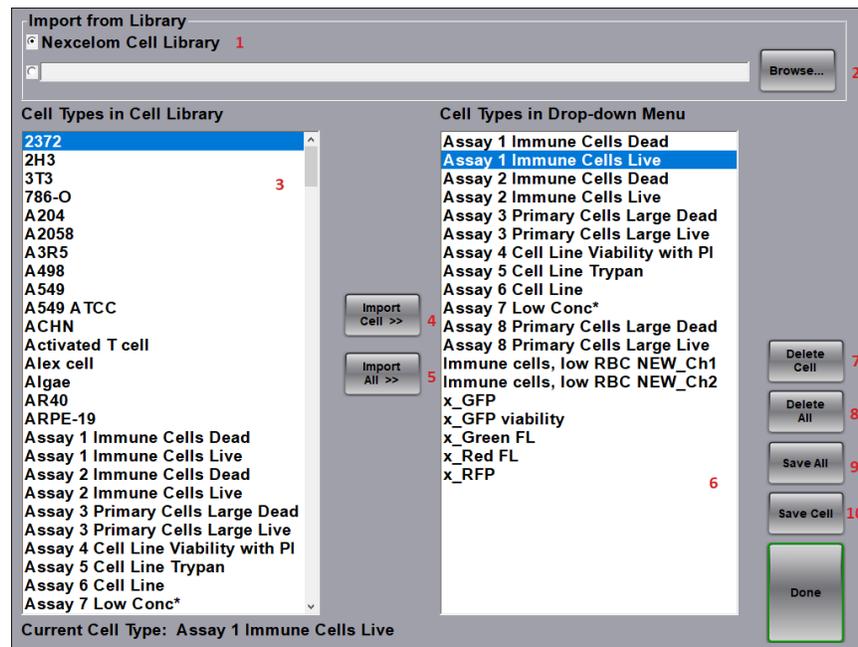
1	Selects built-in **Nexcelom Cell Library** to display cell types in the *Cell Types in Cell Library* pane.
2	Selects a custom library to display available cell types in the *Cell Types in Cell Library* pane. If no library name is displayed, tap the **Browse** button and navigate to an external cell type library.
3	List of cell types in the currently selected Cell Library.
4	Imports the highlighted cell type (from the left pane) into the drop-down menu (right pane).
5	Imports all cell types (from the left pane) into the drop-down menu (right pane).
6	List of cell types to be displayed in the drop-down menu.
7	Deletes the highlighted cell type in the drop-down menu (displayed in the right pane).
8	Deletes all cell types in the drop-down menu (displayed in the right pane).
9	Saves all cell types to a library file (e.g., *MyCells*) in the specified network location.
10	Save the highlighted cell type to a library file (e.g., *MyCells*) in the specified network location.

Cell Type Drop-down Defaults

The Auto 2000 displays *all* standard cell types from the built-in Cell Library in the *Cell Types in Drop-down Menu* pane of the Import Cell Type screen as initial defaults. Users can import additional custom cell types to appear in this drop-down as necessary from an external network location.

Note: If cell types contained in the Cell Library can no longer be found (i.e., they have been cleared by users over time), they may be imported again from either the built-in Cell Library or an external library as indicated below. See the *Cell Library* on page *74* for a list of all available standard cell types.

Importing Cell Types

Used to import cell types from the built-in Cell Library or from external custom libraries. Individual cell types can then be selected to appear in the *Cell Types in Drop-down Menu* pane.

FROM BUILT-IN CELL LIBRARY

The built-in **Cell Library** is available by default and if selected in the Import Cell Type screen, available cell types are displayed in the *Cell Types in Cell Library* pane.

1. In the *Import from Library* section of the Import Cell Type screen, ensure that the **Nexcelom Cell Library** radio button is selected.

2. In the *Cell Types in Cell Library* pane (displayed on the left), highlight a cell type to be imported.

3. Tap **Import Highlighted >>** to import highlighted cell type to appear in the *Cell Types in Drop-down Menu* pane (displayed on the right).

    Note: Although the **Import All >>** button can be used to import *all* cell types, it is not recommended if the size of the Cell Library will make the *Cell Types in Cell Library* pane difficult to use.

FROM CUSTOM LIBRARIES

1. In the *Import from Library* section of the Import Cell Type screen, tap the custom cell library radio button to choose a custom library (if available) or tap **Browse** to navigate to folder containing a custom library and double-tap on a library name to select it. *This text box will appear as blank if a custom cell library has not yet been selected.*

2. Ensure that cell types from the selected library appear in the *Cell Types in Cell Library* pane.

3. In the *Cell Types in Cell Library* pane (displayed on the left), highlight a cell type to be imported.

4. Tap **Import Highlighted >>** to import highlighted cell type to appear in the *Cell Types in Drop-down Menu* pane (displayed on the right).

    Note: If the custom library selected is of a manageable size for the *Cell Types in Drop-down Menu* pane, **Import All >>** can be used to import *all* cell types from the library followed by tapping **Copy** in response to the confirmation prompt.

Deleting Cell Types

1. To delete a single cell type from the *Cell Types in Drop-Down Menu pane* (displayed on the right), highlight the cell type and tap the **Delete Cell** button. To delete *all* cell types from the *Cell Types in Drop-Down Menu* pane, tap the **Delete All** button.

2. In response to the confirmation prompt, tap **Delete** or **Delete All** to confirm the deletion.

3. Confirm cell type has been removed from *Cell Types in Drop-Down Menu* pane.

   Note: If cell type deleted was from the drop-down menu, it can be re-imported if necessary from the built-in cell library. If cell type was from a custom library or created using the Auto 2000, it may be permanently deleted unless cell type was exported/saved prior to deletion.

Saving Cell Types to a File

1. To save a single cell type listed in the *Cell Types in Drop-down Menu* pane (displayed on the right), highlight the cell type and tap the **Save Cell** button. To save *all* cell types from the *Cell Types in Drop-Down Menu* pane, tap the **Save All** button.

2. Select an **Available Drive** (displayed in bottom left corner of the screen) and then navigate to a network location by tapping folder icons.

3. Choose an existing file or tap **New File** to create a file. *If necessary, create a new folder before creating a file.*

4. Use the virtual keyboard to enter a file name and tap **Done**.

5. If saving a single cell type, tap **Continue** in response to confirmation prompt indicating file name to be saved. If saving *all* cell types, indicate whether to *Lock the cells from future editing* by tapping **Lock** or **Unlock**, followed by **Continue** in response to confirmation prompts.

6. Tap the **Done** button to return to the Channel 1 Settings screen.

BUILT-IN CELL LIBRARY

The complete collection of cell types available for import from the built-in Cell Library is listed below.

2372	CL-1	HL60	MC32A-B7
2H3	Colo205	HL-60	MC-38
3T3	Cortical neurons	HL-60 (TB)	MC-38-B7
786-O	Cos7	HMN	MCF-10A
A204	CRL-1863	HNPM	MCF-10A4
A2058	CT-26	HOP-62	MCF7
A3R5	CT-26-B7	HOP-92	MCF7 clumpy
A498	CT-26-CEA	HOS	MCF7 non-clumpy
A549	CT26-CEA-B7	HOS MNNG	MCF7_clumpy
A549 A TCC	D cell	HPAF II	MDA-231
ACHN	D Mel	HS 578T	MDA-MB-231
Activated T cells	D1.1	HT1080	MDA-MB-231 (A TCC)
Alex cell	DC145	HT-1080	MDA-MB-435
Algae	DC-PBMC mix	HT-29	MDA-MB-468
AR40	Dictyoslelium	Human B cell	MDBK
ARPE-19	DLD	Human T cell	MDCK-Focus
Assay 1 Immune Cells Dead	D-MEL	HUT78	medium size cells
Assay 1 Immune Cells Live	DU	HUVEC	MEF
Assay 2 Immune Cells Dead	DU145	Hybridoma	Melan A
Assay 2 Immune Cells Live	DU-145	I9.2	Melan-3m
Assay 3 Primary Cells Large Dead	DU145 G37	IGROV1	Melanocyte
Assay 3 Primary Cells Large Live	EKVX	Initial Cell Type	Melanoma
Assay 4 Cell Line Viability with PI	EL4	Ink-4A	Mel-STR
Assay 5 Cell Line Trypan	Fao	INS	Mel-STV
Assay 6 Cell Line	Fibroblast_human	K1	MES-SA
Assay 7 Low Conc*	Fibroblast_human_high density	K562	MG63
Assay 8 Primary Cells Large Dead	Fibroblast_pig	K9 fibroblast	MM1S
Assay 8 Primary Cells Large Live	Focus	KA T4	MMS
Astrocyte	FRhK4	KM12	MNT1
aTC6	FU5AH	KR158	MOLT-4
B cell_human	G37	KT1	Monocyte
B16	GM cell	KT-1 cell	Mouse ES cell
BHK	H157	KT2	Mouse ES feeder cell
break	H358	KT2 cell	MRC5
BT	H42E	KU812	MSB1
BT-549	H520	L cell	MTA-9
BXPC3	H647	L1210	MV411
C2C12	H9	L929	N59
C6	HASMC	large size cells	NCI-460
C6-36	HBL-2	LCL	NCI-ADR-RES
CAKI-1	HC9	LLC-PK1	NCI-H226
CALU6	HCC-2998	Lncap	NCI-H23
CCR5	HCE4	LOX IMVI	NCI-H322M
CCRF-CEM	HCT-116	Lymphoblast	NCI-H460
CD14	HCT15	Lymphoma	NCI-H522
CD3	HCT-15	M14	NCI-H929
CD3_high concentration	HDF	M19	ND468
CD4	HEC	Macrophages	Neutrophile
CD8 clone	HEK293	MAGIC	new cell type
CEF	HEK293T	Malme 3M	NIH 3T3
CGN	Hela	Malme-3M	NM4A
CHO-1	Hi5	Mast	NM4A4
CHO-3	High 5	MC32A	OCIM1

OVCAR	RCC45	SN12C	U251-c
OVCAR-3	RCS	SNB-19	U266
OVCAR-4	RD	SNB-75	U2OS
OVCAR-5	RD cell	SNU475	U87
OVCAR-8	ReN	SR	U937
P19	RPMI 8226	SW48	UACC 257
PA-317	RPMI7951	SW480	UACC-257
PC	RPMI-7951	SW620	UACC-62
PC12	RXF 393	T cell	UO-31
PC-12	SaOS	T cell_human	V12
PC3	SF-268	T-47D	V529
PC-3	SF-295	TH1	Vero
PDAC	SF-539	THP	WIDR
PLHC	SF9	Til-T	Wil2S
Primary breast-cancer cell	SK28	TK-10	x_GFP
Primary breast cell	SKMel28	TME-7	x_GFP viability
Primary chondrocytes	SK-MEL-28	Tn5	x_Green FL
QNR-K22	SK-MEL-5	Training Beads	x_Red FL
Raji	SKOV3	TS	x_RFP
Ramos	SK-OV-3	U118	
RAW	small size cells	U251	

If you would like help creating a new assay, customizing cell type parameters for an assay or creating a new cell type, send saved raw images to CellC-support@revvity.com and we will optimize a custom assay/cell type for you. See *Saving Count Results* on page *25* for details on how to save information that Revvity will need.

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Chapter 12. User and Instrument Settings

This chapter describes Auto 2000 *Counting Options* and *Saving Options* user settings. See *Taking a Background Image* on page *15* for instructions on using the *Take Background Image* instrument setting.

Tap the **Settings** icon from the Home screen to manage user and instrument settings. Note that some settings in this screen are intended for use only by Support or your IT team. For example, the *Service Options* instrument setting is intended *For Advanced Users Only*, while the *Windows Explorer* and *Windows Control Panel* computer settings are *For IT Professional Use*.
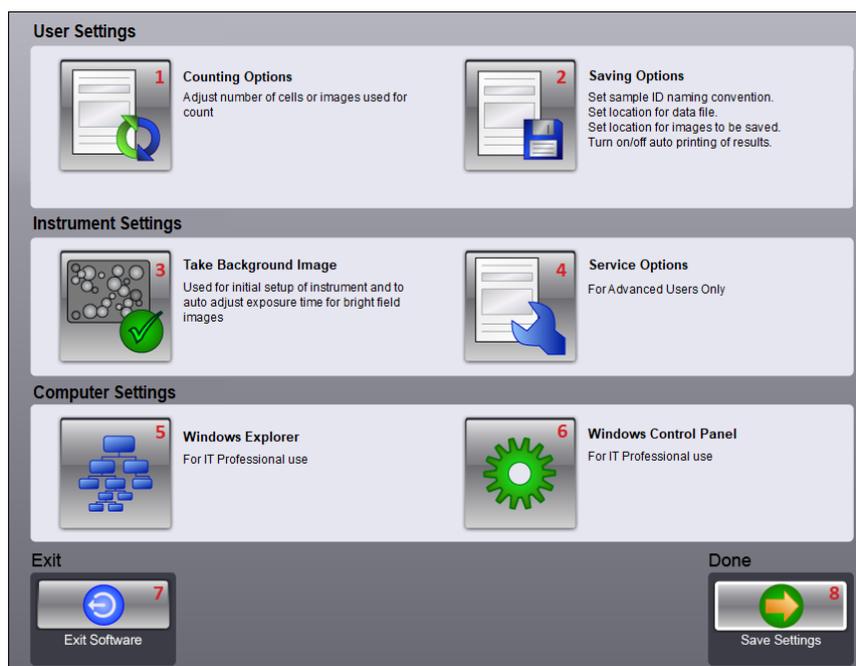
1   **Counting Options** – Allows users to specify counting options (*Count All* or *Speed Count* by limiting the number of cells or images).

2   **Saving Options** – Allows users to modify save options such as Sample ID naming convention, set default locations for data and image files, and enable/disable the *Auto Save* and *Auto Print* features.

3   **Take Background Image** – Allows users to take a background image the first time the instrument is used or if the system is moved to a new location. See *Taking a Background Image* on page *15*.

4   **Service Options** – Hardware settings that are password protected for use *only* by Support.

5   **Windows Explorer** – Allows access to Windows Explorer. *For IT Professional Use.*

6   **Windows Control Panel** – Allows access to Windows Control Panel. *For IT Professional Use.*

7   **Exit** – Closes the Cellometer Auto 2000 software and returns user to Windows desktop.

8   **Done** – Saves any changes to user, instrument and computer settings and returns to the Home screen.

COUNTING OPTIONS

Tap the **Counting Options** icon on the Instrument Settings screen to modify counting options. You can select either the *Count All* or *Speed Count* option in the User Settings screen.

Note: Multiple images are taken of a sample during the counting process and are accessible when using the *Current Image Control* as images *A*, *B*, *C* and *D* (representing counting chamber fields of view). See the *Count Details Screen* on page *23* for details on switching between the display of these images in the viewing pane.

The **Count All** option is enabled by default. This option counts *all* cells in *all* images of the sample.
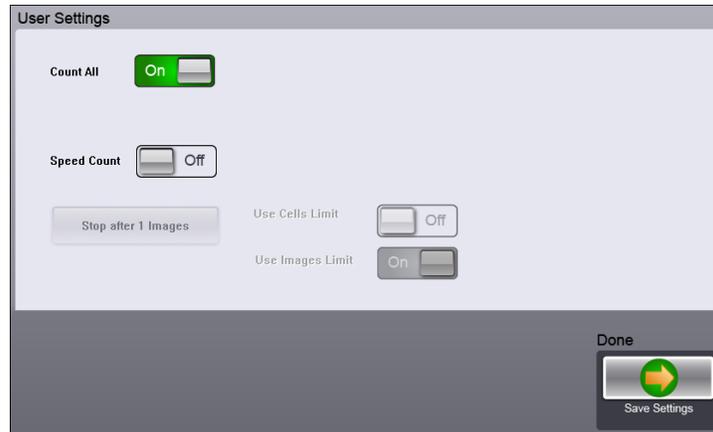
To enable speed counting, tap the **Speed Count** option and choose a parameter to limit the count: *Use Cells Limit* or *Use Images Limit*. Tap on the **Stop after…** button and use the virtual keypad to enter a number of cells or images to stop the counting process accordingly.
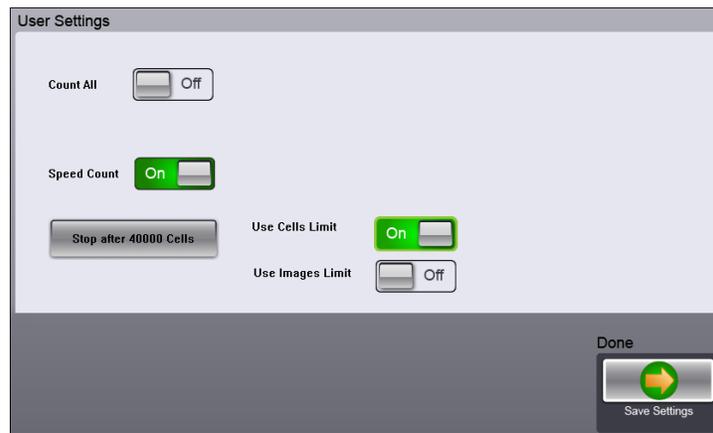
Note: The *Use Cells Limit* parameter defines maximum number of cells to be counted in an image before moving on to the next image, whereas the *Use Images Limit* parameter defines the number of images to be counted (e.g., if a value of *2* is entered, only cells in the first two images will be counted). *All four images of the sample will be taken regardless of Counting Options selected.*

Tap **Done** to save the current *Counting Options* user settings and return to the Instrument Settings screen.

SAVING OPTIONS

Tap the **Saving Options** icon on the Instrument Settings screen to modify save options such as Sample ID naming convention, set default locations for data and image files, and enable/disable the *Auto Save* and *Auto Print* features.

Save Options

1. **Set Sample ID as Cell Type** – Inputs a Sample ID default that matches the cell type being counted (e.g., *"Pan 02"* will be displayed for the *Pan-2* cell type). *This parameter cannot be selected until the Auto Increment Sample ID is enabled to allow automatic incrementing of Sample IDs.*

2. **Auto Increment Sample ID** – Appends the Sample ID default with an incremented numerical value (e.g., *Pan 02_001, Pan 02_002,* etc.).

3. **Log User Name** – Requires entry of a User ID as well as a Sample ID to be saved with the data. *Appends a User ID column to the data text file. Once a User ID is entered, it will remain as the default until changed.*

4. **Time Stamp Sample ID** – Appends Sample ID with a date/time stamp of when count was performed.

5. **Include Instrument ID in File** – Saves Instrument ID on which the count was performed with count results. *Appends an Instrument column to the data text file.*

Auto Save Options

6. **File:** *file_name* **Button** – Allows users to specify the file path and name of the text file to be used for saving count result data. *The default data file name will be either data.csv (i.e., indicating a plain text file consisting of comma-separated values that can be used with a spreadsheet application) or data.txt (i.e., indicating the plain text file historically used by Cellometer Auto 2000).*

7. **Auto Save to Data File** – Enables automatically saving of data to text file after a count is performed.

8 **Create New File for Each Sample** – *Requires **Auto Save to Data File** to be enabled.* Creates a new data text file each time cells are counted.

9 **Folder:** *file_path* **Button** – Allows users to specify the folder to be used for saving image files captured during a count. *It is recommended that image files be stored on the network or USB drive.*

10 **Save Raw Images** – Enables automatic saving of raw images (.pngs) to the folder specified. *Only raw images can be loaded into the Auto 2000 for reanalysis or sent to Support when requesting assistance with optimization of assay/cell type parameters.*

11 **Save Counted Images** – Enables automatic saving of counted images (.jpgs) to the folder specified. *Selecting this option saves two copies of each image; one image with the counted graphic overlay (i.e., "Counted" appears in the file name) while the other image displays cells without the graphic overlay.*

Auto Print Options

12 **Auto Print Results** – Enables automatic printing of count results after completion of each count. *If a printer is not connected to the instrument, you must also enable the **Print with Default Printer** option.*

13 **Print with Default Printer** – Enables use of the Windows default printer when automatically printing count results.

Save Settings

14 Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Defining Default Data File/Image Folder Locations

Users can save count results as necessary by tapping the **Save Copy of Data** icon on the Count Details screen. Follow these steps to define the default location for data and image files.
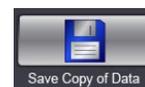
Note: The Auto Save feature does *not* need to be enabled in order to define default locations to be used when saving count result data and image files while viewing individual samples.

1. From the Home screen, tap the **Settings** icon.

2. In the *User Settings* area, tap the **Savings Options** icon.

3. In the *Auto Save* area of the screen, tap the **File:** *file_name* button to specify the file path and text file to be used for saving count result data. *The default data file name will be either data.csv (i.e., indicating a plain text file consisting of comma-separated values that can be used with a spreadsheet application) or data.txt (i.e., indicating the plain text file historically used by Auto 2000).*

   Navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**.

4. Select the **Folder:** *file_path* button to specify the folder to be used for saving image files captured during a count. *Due to the size of image files, it is recommended they be stored on a USB drive or a network. Request help from your IT team before using the **Windows Explorer** option in the Computer Settings area to map available drives to your internal corporate network.*

5. Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Establishing a Windows Default Printer

Users can send count results to a printer as necessary by tapping the **Print** icon from the Count Results screen. Follow these steps to connect to a default Windows printer. *If a printer is connected to the instrument with a USB cable, establishing a default printer is not necessary.*

Note: The Auto Print feature does *not* need to be enabled in order to establish a Windows default printer to be used when sending count results to a printer while viewing individual samples.

1. From the Home screen, tap the **Settings** icon.

2. In the *User Settings* area, tap the **Savings Options** icon.

3. In the *Auto Print* area of the screen, tap the **Print with Default Printer** button to display as *ON*. *Request help from your IT team before using the **Windows Control Panel** option in the Computer Settings area to set up a Windows default printer.*

4. Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Setting Up Auto Save Feature

Follow these steps to set up automatic saving of count results each time you perform a count.

1. Insert a USB drive into the instrument's USB port or connect an Ethernet cable to link instrument to a network.

2. From the Home screen, tap the **Settings** icon.

3. In the *User Settings* area, tap the **Saving Options** icon.

4. In the *Auto Save* area of the Saving Options screen, tap the **Auto Save to Data File** button to display as *ON*.

5. Tap the **File:** *file_name* button to specify the file path and text file to be used for saving count result data. *The default data file name will be either data.csv (i.e., indicating a plain text file consisting of comma-separated values that can be used with a spreadsheet application) or data.txt (i.e., indicating the plain text file historically used by Auto 2000).*

   Navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**.

   Note: File name entered is used when Auto Save feature is enabled *as well as* when manually saving count results using the **Save Copy of Data** icon on Count Details screen. See *Saving Count Results* on page *25*.

6. To create a new file for each sample, tap the **Create New File for Each Sample** button to display as ON. *Keep in mind that selecting this option may result in a large volume of files.*

7. Select the **Folder:** *file_path* button to specify the folder to be used for saving image files captured during a count. *Due to the size of image files, it is recommended they be stored on a USB drive or a network. Request help from your IT team before using the **Windows Explorer** option in the Computer Settings area to map available drives to your internal corporate network.*

Navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**.

Note: Folder name entered is used when Auto Save feature is enabled *as well as* when manually saving count results using the **Save Copy of Data** icon on Count Details screen. See *Saving Count Results* on page *25*.

8.   Indicate the type of image files to be saved automatically by tapping the **Save Raw Images** and/or **Save Counted Images** buttons to display as *ON. Only raw images can be loaded into the Auto 2000 for reanalysis or sent to Support when requesting assistance with optimization of assay/cell type parameters. Selecting Counted Images saves two copies of each image; one image with the counted graphic overlay (i.e., "Counted" appears in the file name) while the other image displays cells without the graphic overlay.*

9.   Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Setting Up Auto Print Feature

Follow these steps to set up automatic printing of count results each time you perform a count.

1.   From the Home screen, tap the **Settings** icon.

2.   In the *User Settings* area, tap the **Savings Options** icon.

3.   In the *Auto Print* area of the screen, tap the **Auto Print Count Results** button to display as *ON. This enables automatic printing of count results to a printer connected to the instrument with USB a cable.*

4.   To enable printing to the Windows default printer on your network, tap the **Print with Default Printer** button to display as *ON. Request help from your IT team before using the **Windows Control Panel** option in the Computer Settings area to set up a Windows default printer.*

   Note: If **Print with Default Printer** is enabled, count results will be sent to the Windows default printer when the Auto Print feature is enabled *as well as* when manually printing count results using the **Print** icon on the Count Results screen.

5.   Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Chapter 13. Cleaning, Maintenance and Storage

Keeping the Auto 2000 and its operative area clean between runs, during use and post runs is a best practice and prevents contamination. Caring for the instrument and its consumables is also a best practice.

Note: If using the Auto 2000 within a biosafety cabinet, cleaning may not be required or agents and materials may be adapted according to BSC system requirements. Please follow all the instructions provided by the manufacturer.

⚠️ **CAUTION:** Always power the instrument OFF before cleaning as damage to the machine could occur.

⚠️ **CAUTION:** Allow for flammable agents used for cleaning or disinfecting (or as solvents of adhesives) to completely evaporate before powering the instrument ON.

CLEANING

The instrument and any cords/cables can be wiped down using a 70% Isopropyl (IPA) solution. Repeat until the soil is no longer visible. Finish with a fiber optic lint-free wipe (e.g., Kimwipes).

1.   Dampen a fiber optic lint-free wipe with IPA.
2.   Use the wipe to rub lightly on the outside of the instrument until it is visibly clean.
3.   Wait for the cleaning agent to evaporate before powering the instrument ON.

Should something break or be spilled inside the device, power OFF the instrument and contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

ROUTINE MAINTENANCE

No one other than Revvity-authorized personnel may service inside the protective instrument cover of the Cellometer Auto 2000. Contact Support or an authorized service representative to address any changes in instrument output or performance.

⚠️ **WARNING:** Do not remove the instrument cover due to an electric shock hazard. Contact Support for assistance.

Contacting Support

All technical questions regarding maintenance should be directed to Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Preventive Inspection and Maintenance

Regular preventive inspections should be carried out to reduce safety concerns of the instrument due to aging, normal wear and tear, etc. The manufacturer assumes no responsibility for improper changes or repairs carried out on the instrument or its accessories by unauthorized persons. The warranty will immediately become void should an unauthorized personnel attempt to in any way repair or modify the instrument.

Contact Support to schedule all preventive maintenance needs and address any functionality concerns by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

STORAGE

When preparing the instrument for storage:

- Always thoroughly clean the instrument, cables and any of the accessories or consumables before storage.
- Check for any damage and if possible, re-package the Instrument and Operating Computer.
- Ensure that storage temperature and spatial requirements are met (see *Site Preparation* on page *14*).
- DO NOT put anything heavier than ≥25 lbs. on the instrument or the box in which it is stored.

Store the cables neatly, checking for any signs of damage or wear frequently and immediately before/after use. Do not allow the cables to become kinked or tangled. Do not set heavy objects on any of the accessories or consumables.

Always store beads and reagents according to their information for use documentation.

Chapter 14. Troubleshooting and FAQs

This chapter lists troubleshooting steps for resolving potential issues, common instrument messages and *Frequently Asked Questions* (FAQs).

TROUBLESHOOTING AND INSTRUMENT MESSAGES

Particles appear in the background/background as too dark

Take a new background image by following the steps presented in *Taking a Background Image* on page *15*.

Instrument cannot be powered on

- Check to ensure the Power Cord that was provided with the instrument is being used.
- Check Power Cord to confirm it is not kinked or tangled.
- Check that Power Cord is plugged in properly to both the outlet and the instrument.
- Check that Power Switch on instrument displays *Blue* indicating power is *ON* (in *Run* mode) and not *Orange* indicating power is *OFF* (in *Standby* mode).

Instrument displays "Could not read GPIO Values", "*Error setting up Preview*" and "*No instrument detected. Running software in data analysis mode*" messages

1. Power OFF the instrument.
2. Remove all USB devices (e.g., mouse, keyboard, USB drive).
3. Unplug the instrument Power Cord from the electrical outlet (wait 5 seconds).
4. Plug the instrument Power Cord into the electrical outlet.
5. Power the instrument ON (with no USB devices attached).
6. Once the instrument is fully booted, reconnect USB devices.

If the problem persists, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Instrument displays "*Background was saved, but … Dim XXX counts*" message

Contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

FREQUENTLY ASKED QUESTIONS

Can SD025 slides be used on the Auto 2000?

No, SD025 slides are intended for use with small cells (such as bacteria) in higher magnification systems.

How do I upgrade to the newest version of the software?

Contact Support with the serial number of the instrument to request an upgrade.

How do I order a replacement for an instrument Power Cord that has been lost?

Contact Support with the serial number of the instrument to request a replacement.

Why doesn't the cell type I need appear in the Cell Type drop-down?

Cell types can be imported from either the Cell Library or from custom libraries stored in external network locations. See *Importing Cell Types* on page *71* for instructions on how to import a cell library and populate the Cell Type drop-down menu with selected cell types. Contact Support for a full list of available cell types and/or if you require a specific cell type.

When testing for cell viability, why are my count results lower than expected?

When preparing a sample to test for cell viability, ensure that the stock solution for any stain being used is as indicated in *Staining Solution Guidelines* on page *33*. Using stains in a concentration *higher* than recommended concentrations will make the cells more difficult to detect and may result in counting inaccuracies.

In addition, ensure that:

- The viability method is appropriate for the cell type. See *Evaluating Viability Methods* on page *31* for details.
- The current assay is appropriate for the cell type. See *Default Assays* on page *47* for the intended use of default assays.

Chapter 15. Contacting Support

This chapter presents the scope of Support services and provides contact methods. In addition, it contains instructions on how to report issues to Support.

SCOPE OF SUPPORT SERVICES

Revvity is dedicated to providing our customers with outstanding support including the following services:

- Online and in-lab customer training
- Creation of new cell types
- Optimization of counting parameters
- Troubleshooting via telephone
- Periodic safety checks and functional evaluations (offered as part of a separate maintenance contract)

To inquire about training, visit our website at https://www.revvity.com/contact-us/customer-training and choose the *Cell Counting and Image Cytometry* service. Enter your contact details and any training comments/questions.

CONTACT METHODS

If there is a technical issue with your instrument or software, contact Support using the following methods:

- Visit https://www.revvity.com/contact-us/instrument-support-and-service and choose the *Cell Counting and Image Cytometry* product for support. Enter instrument serial number, name and model, your contact details, and a detailed description to report the issue to Support.

- Visit https://www.revvity.com/contact-us-by-phone to find the global phone number for your area.

- Send email to CellC-support@revvity.com

REPORTING AN ISSUE TO SUPPORT

If an issue encountered *cannot* be resolved using troubleshooting steps presented in this guide (see *Troubleshooting and Instrument Messages* on page *85*) or the issue persists after rebooting the instrument, perform the following steps before creating a Support ticket:

1. Record the error message.
2. Record the sequence of events that caused the error, if possible.
3. Close the error message window.
4. Record the Serial Number located on the Device Label for your instrument.

Gathering these details *prior* to contacting Support will be helpful as they troubleshoot the technical issue.

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Appendix A. Consumables

This appendix presents Revvity consumables designed specifically for the Auto 2000 such as disposable counting chamber slides, reagents/reagent kits and counting beads.

COUNTING CHAMBER SLIDES

Cellometer *Counting Chamber Slides* are compatible for use with all Cellometer systems. Each all plastic, disposable slide contains two sample counting chambers with precisely controlled height. The fixed 20 µL sample size allows for simple, automated calculation of cell concentration following imaging and counting.

Image-based counting with disposable counting chambers offers several key advantages:

- No potential clogging
- Ideal for fragile samples, such as hepatocytes
- No washing
- No potential cross-contamination

Visit the *Cellometer and Cellaca Slides* page on our website for a current listing or contact your Sales representative to purchase Cellometer counting chamber slides directly from Revvity.

REAGENTS AND KITS

Revvity offers a variety of reagents and reagent kits to accurately perform fluorescence-based cell counting and viability assays, including measuring percent viability and the number of live/dead cells.

Visit the *Reagents and Kits for Cell Counting and Cell-Based Assays* page on our website for a current listing or contact your Revvity Sales representative to purchase Cellometer assay reagents and kits directly from Revvity.

COUNTING BEADS

Revvity offers a wide range of brightfield and fluorescent *Counting Beads* that may be used to verify instrument functionality and establish routine quality control SOPs for daily, weekly or monthly performance. *Beads are not intended to replace certification by Installation/Operation Qualification (IQ/OQ) procedures.*

Beads are supplied with the proper protocol, a *Certificate of Analysis*, and pass/fail criteria where users may choose to test concentration or viability read-outs on their instruments.

Visit the *Cellometer and Cellaca Counting Beads* page on our website for a current listing of *Check Validation Bead Solution* and *Polystyrene Beads in Trypan Blue* or contact your Sales representative to purchase counting beads for use with Cellometer instruments directly from Revvity.

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Appendix B. Warranty and License Details

This appendix presents *Warranty Information* for Cellometer instruments, Revvity's *Limitation of Liability (Hardware and Software)* statement, and *Terms and Conditions* related to the use of Cellometer software and related documentation. In addition, it includes a definition of *Revvity's Proprietary Information*.

WARRANTY INFORMATION

Revvity warrants that Cellometer instrumentation products shall, for a period of twelve (12) months from the date of purchase, be free of any defect in material and workmanship. The sole obligation of this warranty shall be to either repair or replace at our expense the product, at manufacturer's option. The original sales receipt must be supplied for warranty repair. Products which have been subjected to abuse, misuse, vandalism, accident, alteration, neglect, unauthorized repair or improper installation will not be covered by warranty.

Instruments must be handled and packaged correctly when shipping to other locations. Contact Revvity for additional information and to order packaging materials.

Any product being returned is to be properly disinfected and packaged (in original packing if possible). Damage sustained in shipping due to improper packing will not be covered by warranty.

TERMS AND CONDITIONS

The *Revvity, Inc. – Terms and Conditions of Sale* license agreement states the terms and conditions upon which Revvity Bioscience offers to license to you the software together with all related documentation. The Software is licensed to you for use only in conjunction with Revvity's family of products.

In addition, the original Cellometer software and any software upgrades installed on your Cellometer system by authorized representatives of Revvity is protected. You may not tamper with this software (including unauthorized upgrades), disclose it to third parties or use it for any purpose other than running your Cellometer system. Revvity does not grant you any other rights to use or disclose the original Cellometer software or upgrades, and any further uses will be prosecuted by Revvity to the maximum extent possible by law. Any other use of Cellometer software or upgrades is explicitly prohibited. In addition, you may not disclose Cellometer software, upgrades, or any of its features and benefits to a third party.

Revvity Proprietary Information

Cellometer products have been developed by Revvity and include certain intellectual property of Revvity, including without limitation, software, samples, schematics, specifications, manuals, designs, and other technical, business, trade secret, proprietary and confidential information provided to Buyer by Revvity ("Revvity Proprietary Information").

Buyer is granted a non-exclusive right and license to use the Revvity Proprietary Information solely: (a) as incorporated into, and in conjunction with, the products, (b) in conformance with the specifications, and (c) for Buyer's internal use.

Buyer may not: (i) assign, sublicense, transfer, lease, rent or distribute any of its rights in the Revvity Proprietary Information; (ii) port, translate, localize or create derivative works based upon the Revvity Proprietary Information in any manner; (iii) reverse assemble, decompile, reverse engineer, translate or otherwise attempt to derive or obtain the source code, the underlying ideas, algorithms, structure or organization of the Revvity Proprietary Information; (iv) use the Revvity Proprietary Information for the benefit of any third party including as part of any service bureau, time sharing or third party training arrangement; or (v) publish any benchmark testing results on any product or the Revvity Proprietary Information without Revvity's written consent.

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Buyer shall use reasonable care to protect the Revvity Proprietary Information, and in no event less than the care Buyer uses to protect its own like information.

LIMITATION OF LIABILITY (HARDWARE AND SOFTWARE)

Cellometer® automated cell counting instruments, software and consumables are intended for research use only.

In no event shall Revvity be liable for any damages whatsoever (including, without limitation, incidental, direct, indirect, special or consequential damages, damages for loss of business profits, business interruption, loss of business information) arising out of the use or inability to use this Software, Consumables or related Hardware.

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