USER MANUAL

CELLOMETER® SPECTRUM

8001505 Rev D

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

Cellometer® Spectrum User Manual

8002632 Rev D

February 2024

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Chapter 1. Introduction

This chapter describes the Cellometer Spectrum instrument, provides quick operation instructions and presents an overview of the software, terminology and screen elements used.

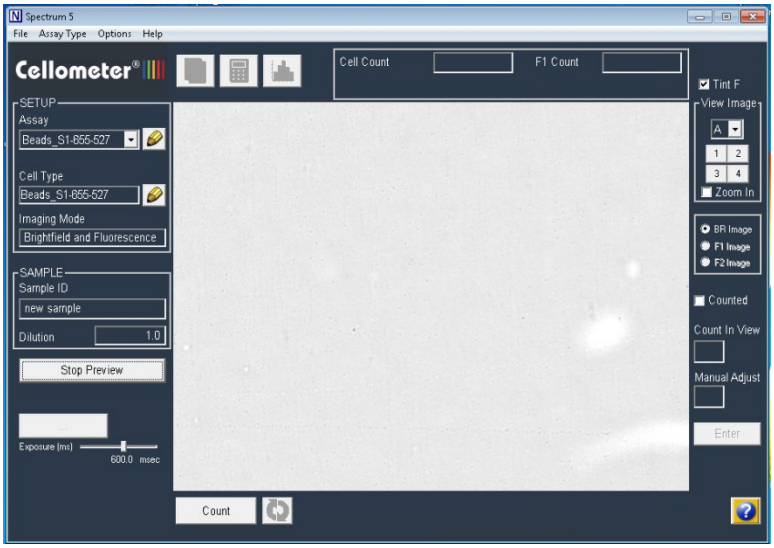
CELLOMETER SPECTRUM OVERVIEW

Cellometer® Spectrum is a compact, automated cell analysis system that can count cells, measure cell sizes and detect fluorescent properties of cells.

The basic principle of the Spectrum automatic cell counter is *Imaging Cytometry*. Cells are loaded into the *Disposable Counting Chamber* and automatically spread into a thin layer by capillary action. The Spectrum then captures images of cells in the counting chamber, analyzes the number of cells, sizes and fluorescent intensity of each cell, and then converts this data into concentration, size, and fluorescent histograms and scatter plots.

The Cellometer Spectrum system consists of three main components:

- Cellometer Spectrum Instrument
- Cellometer Spectrum Software and Controller Computer (included with instrument and pre-loaded with software)



- Disposable Counting Chambers

Cellometer disposable counting chamber accommodates two individual samples and can be loaded through either port. Pipette 20µL into one of the ports with any standard single channel pipette. Cellometer Spectrum comes with a starter set of 75 slides. Slides can be ordered directly from Revvity or your authorized Revvity dealer. See *Disposable Chambered Slides* on page *49* for details.

OPERATING COMPUTER MINIMUM REQUIREMENTS

If a laptop was purchased from Revvity along with the instrument (*laptop purchase is optional*), it will be shipped in a separate box. This laptop will have the specifications listed below:

- Windows 10
- Intel® i5 (1.6 - 4.40 GHz) Processor
- 10 Core
- 4 GB RAM
- Integrated Graphics Card
- 1080p Display Resolution
- 500 GB Hard Drive
- USB 2.0 Port

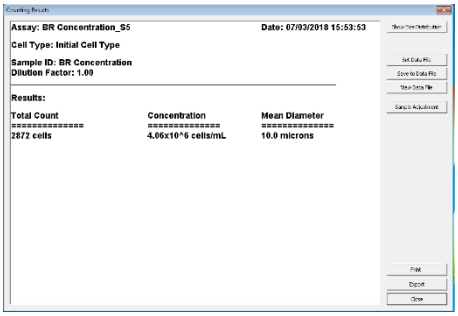
If a laptop was *not* purchased from Revvity, the Operating Computer set up to run with the Cellometer Spectrum instrument must meet or exceed the specifications listed above.

GENERATING DATA WITH CELLOMETER SPECTRUM

Generating cell sample information is quick and easy. Once the instrument is set up for your specific assay, you only need to perform three basic steps to get results:

1. Prepare sample and load counting chamber.

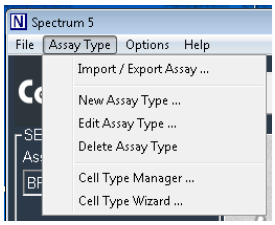
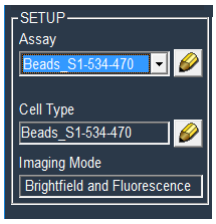
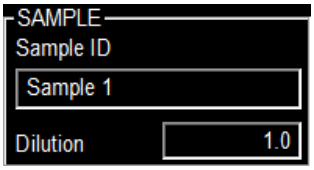
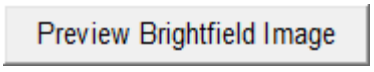
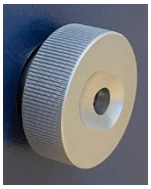
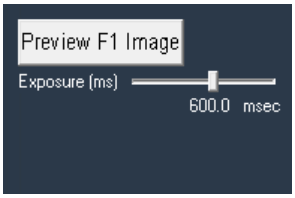
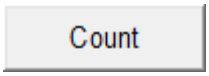
2. Insert counting chamber into Spectrum instrument.

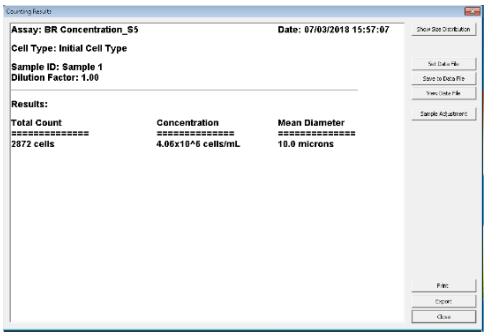


3. Use software to acquire images, analyze samples and view results.

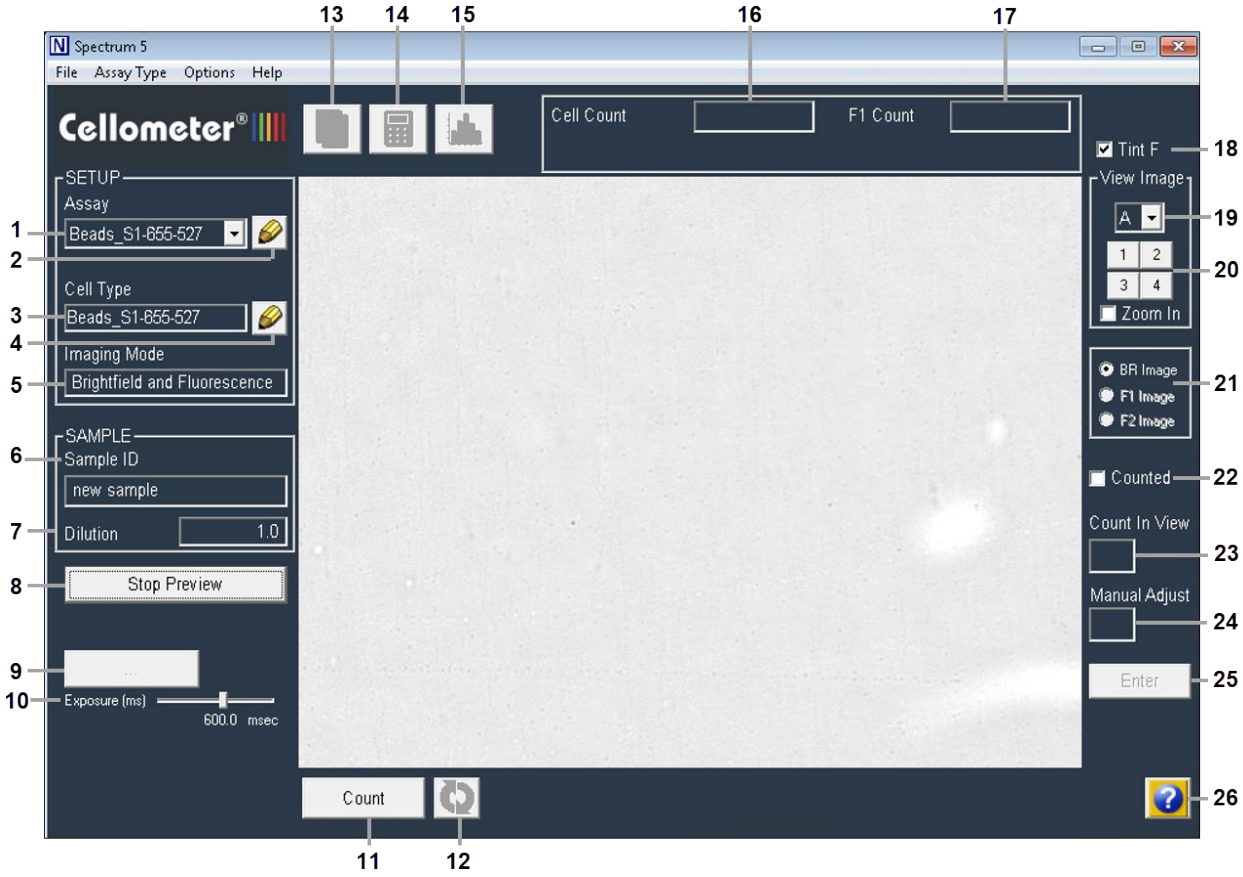
QUICK OPERATION INSTRUCTIONS

The following table presents basic steps to run any assay.

Step	*Instructions*	
1	**Set up or select an assay.** (a) Import or set up a new assay from the Assay Type menu, (b) Select an existing assay from the drop-down or (c) Edit an existing assay.	 **(a) and (c)**  **(b)**
2	**Input Sample ID and Dilution Factor.**	
3	**Prepare sample, load disposable counting chamber and insert into instrument.**	
4	**Click 'Preview Brightfield Image' and ensure image is in focus.**	
5	**Adjust focus.** Slowly turn the focus wheel until optimal cell counting focus is achieved. *Live cells and training beads will have bright centers and clearly defined edges.*	
6	**Click 'Preview F1 Image' and adjust fluorescent exposure time if necessary.** If using Spectrum Trio in dual fluorescence mode: **Click 'Preview F2 Image' and adjust exposure time if necessary.**	
7	**Click 'Count' to begin counting process.**	

Step	*Instructions*	
8	**Review counting results and generate reports.** Counting results will automatically display on screen. Select desired reports/graphs to display, export/print data or click **Done** to clear window and review images.	
9	**Navigate and review images on screen.** Select a section of the image (a) to view, (b) toggle between the brightfield (BR) and fluorescence (F1 or F2) images or (c) manually adjust counted cells.	 **(a)** **(b)** **(c)**
10	If desired, click (a) *Re-display Counting Results*, (b) *Launch Sample Adjustment Calculator* or (c) *Display Size Distribution* icons.	 **(a)** **(b)** **(c)**

SOFTWARE OVERVIEW



Item:	*Name:*	*What it does:*
1	**Select Assay**	Selects a saved assay from the Assay Library.
2	**Edit Assay**	Modifies parameters of currently selected Assay.
3	**Cell Type Display**	Displays the selected cell type.
4	**Edit Cell Type**	Modifies parameters of currently selected Cell Type.
5	**Imaging Mode**	Displays current imaging mode settings.
6	**Sample ID Input**	Allows user to input name/ID/# of current sample.
7	**Dilution Factor Input**	Allows user to input dilution factor.
8	**Preview Brightfield Image**	Previews brightfield image before counting (also Stop Preview).
9	**Preview F1/F2 Image**	Previews the fluorescent F1 or F2 image (based on selection).
10	**F1/F2 Exposure Adjustment**	Adjusts exposure time for F1 or F2 image (in milliseconds).
11	**Count/Speed Count Button**	Initiates counting procedure.
12	**Recount Button**	Recounts currently loaded image.
13	**Display Counting Results**	Opens counting results box.
14	**Sample Adjustment Calculator**	Launches Sample Adjustment Calculator.

Item:	*Name:*	*What it does:*
15	**Display Size Distribution**	Opens size distribution histogram.
16	**Live Cell Count Display**	Shows number of live cells in current view.
17	**F1/F2 Counted**	Shows number of fluorescence positive cells in F1 or F2 image.
18	**Tint F**	Check to display fluorescent cells in false color.
19	**Captured Image Selection**	Toggles view between A, B, C, and D captured images.
20	**Captured Image Quadrant Buttons**	Toggles view between quadrant 1, 2, 3 and 4 of each captured image.
21	**Imaging Mode View Selection**	Toggles view between Brightfield (BR), Fluorescence 1(F1) and Fluorescence 2 (F2).
22	**Counted**	Displays counted images.
23	**Live/Dead Count in View**	Displays total number of live or dead counted cells in view.
24	**Manual Adjust**	Allows user to manually adjust counting results.
25	**Enter**	Updates counting results after entering Manual Adjust values.
26	**Help**	Allows user to access online Support resources. *Requires internet connection.*

TERMS AND ICONS USED

The following terms and icons are used throughout this guide.

Assay	A set of configuration parameters that include the instrument settings (i.e., fluorescence modes, exposure times, counting method and associated cell types).
Cell Type Parameters	A set of configuration parameters specific to a cell used in an assay such as cell size, fluorescence properties and decluster properties.
Count/ Speed Count	The command to initiate an image capture and analysis of a sample in Count/ Speed Count mode.
Decluster	A software function used to distinguish and individually count cells from a cluster of cells.
Brightfield	A standard imaging mode of the instrument using brightfield optics for viewing and counting total cells, measuring cell sizes and determining viability using trypan blue.
Fluorescence	The fluorescent imaging capabilities and modes of the instrument. Used to detect fluorescent properties of cells. *Spectrum Duo has one fluorescent channel while Spectrum Trio has two fluorescent channels.*
Brightfield Image	Image displayed on screen after it has been acquired from the brightfield optics.
Fluorescent Image	Image displayed on screen after it has been acquired from the fluorescence optics.
F1, F2	F1 is the first fluorescent image. F2 is the second fluorescent image.
Threshold	The limit to include or exclude a cell in a particular category (e.g., counted, not counted, live/dead, fluorescent positive/negative, etc.) based on a particular property of the cell (i.e., size, fluorescent level, bright center, etc.).
	**Edit Assay or Cell Type Parameters Icon:** Click to quickly access parameters from the main screen for editing.
	**Generate Data and Reports Icon:** Click to view Counting Results after initial pop-up screen has been cleared.
	**Sample Adjustment Calculator Icon:** Click to display the Sample Adjustment Calculator. Useful for sample adjustment to get desired concentration or total cell number.
	**View Size Distribution Icon:** Click to display size distribution after the initial pop-up screen has been cleared.
	**Recount Icon:** Click to recount the same acquired image after assay or cell parameter settings have been modified.
	**Green Circle in Counted Graphic Overlay:** Indicates a counted cell. In brightfield mode, indicates a live cell when running trypan blue assays. In fluorescence mode, indicates a fluorescence positive cell.
	**Red Circle in Counted Graphic Overlay:** In brightfield mode, indicates a dead cell (trypan blue positive) when running assays. In fluorescence mode, indicates a fluorescence negative cell.

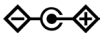
GLOSSARY OF ABBREVIATIONS

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

	**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**		**Korean Certification for EMC Directives and Registration Number. Located on Device SN Label**		**Off – Power Disconnection from Mains. Located on Instrument Power Switch**
	**Serial Number. Located on Device SN Label**		**UK Conformity Assessment Declaration of Conformity to UK Directives. Located on Device SN Label**		**Manufacturer. Located on Device SN Label**

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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 Cellometer Spectrum 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 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 plate at a time. Do *not* attempt to load more than one plate at a time onto the stage. Doing so will cause an error and could damage the instrument.

CAUTION: Do *not* stack equipment on top of the instrument (except for provided laptop) or place the instrument on top of electrical equipment. This is an unstable configuration and does not allow for adequate cooling.

In addition, although instrument comes with vibration minimizing feet, 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 by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Potential Pinching Hazards

The Cellometer Spectrum instrument contains mechanical components that move during operation and pose risk of potential pinching hazards which could cause personal injury.

CAUTION: While an application is executing, keep access door closed as moving mechanical components within the instrument pose risk of pinching, crushing, cutting, twisting or entrapping of body parts. Do not open the access door during operation or personal injury may occur.

Ultraviolet Light Hazard

The Cellometer Spectrum instrument contains highly sensitive optics that use powerful LED and Ultraviolet lights that if looked directly into could cause personal injury.

WARNING: While an application is executing, keep access door closed as the various lights will be activated. Do not open the access door and look into the instrument or personal injury may occur.

Servicing Hazard

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

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 Cellometer Spectrum 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 Cellometer Spectrum instrument to the main power connection *prior* to use. The Cellometer Spectrum must be set, regulated and used in accordance with 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 Cellometer Spectrum 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 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.

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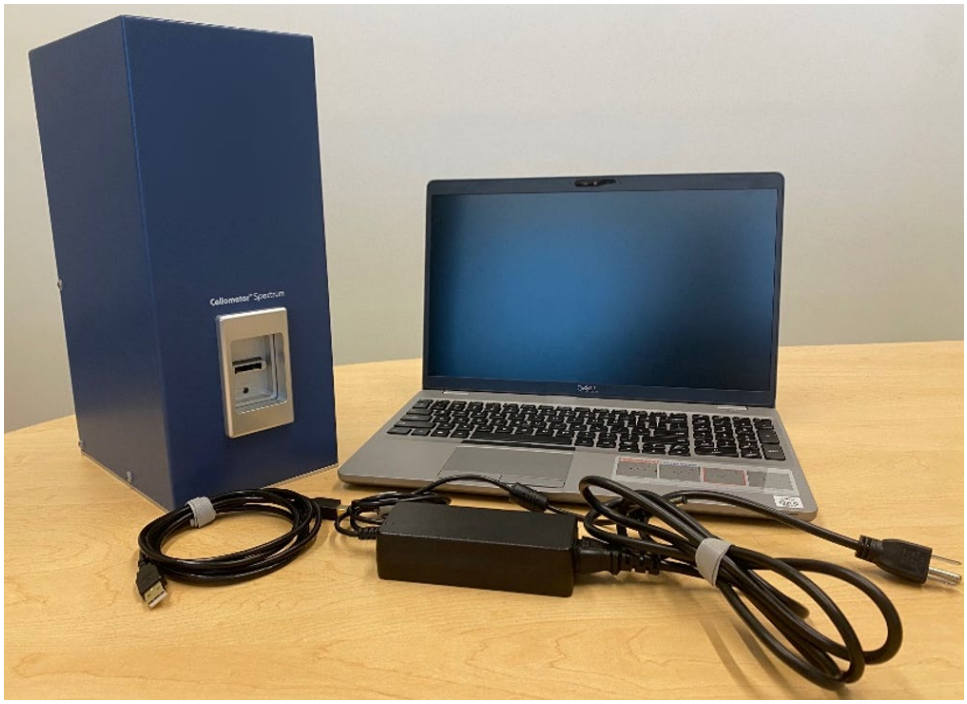
Chapter 3. Getting Started

This chapter reviews Cellometer Spectrum system components, includes setup details and outlines steps for using the instrument for the first time.



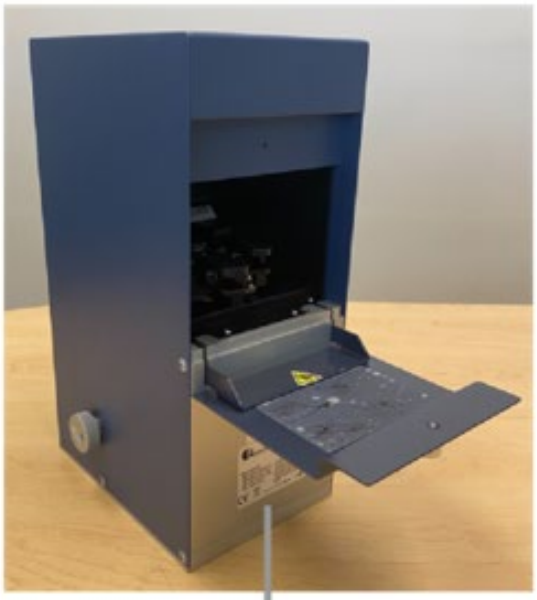
CELLOMETER SPECTRUM SYSTEM COMPONENTS

The Cellometer Spectrum comes with the following components:

- Cellometer Instrument
- Power Supply and Power Cord
- USB 2.0 Connector Cable
- Cellometer Spectrum Software USB Drive (also contains .PDF user documentation)
- User Manual
- Quick Simple Setup Guide
- Quick Start Guide
- Consumable Starter Pack (Disposable Counting Chamber Slides and Bead Solution)
- Revvity-provided Computer Controller and associated accessories (OPTIONAL*)*
  If a laptop was purchased from Revvity along with the instrument, it will be shipped in a separate box. USB Drive is NOT included with laptop as software and user documentation will be pre-installed.)

FRONT VIEW

BACK VIEW



Serial # Label

SETTING UP CELLOMETER SPECTRUM

All Revvity products undergo a rigorous quality inspection prior to shipment and all reasonable precautions are taken in preparing them for shipment to assure safe delivery.

The instrument should be unpacked and inspected for mechanical damage upon receipt. Mechanical inspection involves checking for signs of physical damage such as: broken knobs, scratches, dents, etc.

If damage is apparent or any components are missing, please immediately contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Connecting Spectrum to Computer

After unpacking the instrument and computer (if computer is provided by Revvity), plug the Power Supply and Power Cord into the back of the instrument, and connect instrument to computer using USB 2.0 Connector Cable. **If using a computer that is *not* supplied directly from Revvity (or an Authorized Distributor), the software must be installed BEFORE the USB 2.0 cable is connected.**

Note: Revvity-provided computers are shipped with Cellometer Spectrum software and Microsoft Excel (North America only) pre-installed. No additional setup or configuration is required. If software re-installation is required, contact Support.

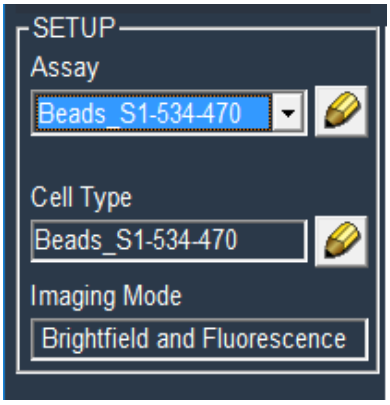
Using Spectrum for the First Time

Before using Spectrum for running samples for the first time, use Bead Solution and Disposable Counting Chamber Slide provided in the Consumable Starter Pack to ensure instrument is set up and configured properly.

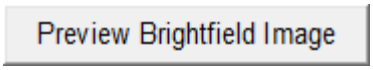
STEP 1. PREPARE SAMPLE SLIDE

- Vortex Bead Solution at low speed for 10 seconds.
- Obtain a clean Disposable Counting Chamber and place it on a clean Kimwipe.
- Pipette a 20µL of solution into one side of the chamber. Capillary force automatically spreads the sample within the counting chamber.
- Hold the loaded chamber in the white area (taking care to stay away from the clear optical window area). Insert chamber into slot in the front of the instrument.

STEP 2. SELECT “BEADS FL1” FROM THE ASSAY TYPE DROP-DOWN MENU.

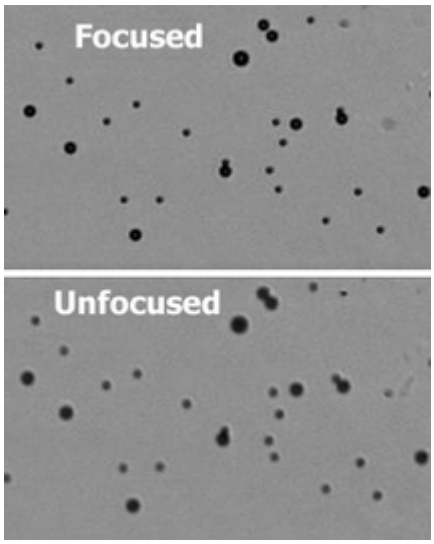


STEP 3. CLICK 'PREVIEW BRIGHTFIELD IMAGE'.



STEP 4. SLOWLY TURN THE FOCUS WHEEL UNTIL OPTIMAL CELL COUNTING FOCUS IS ACHIEVED

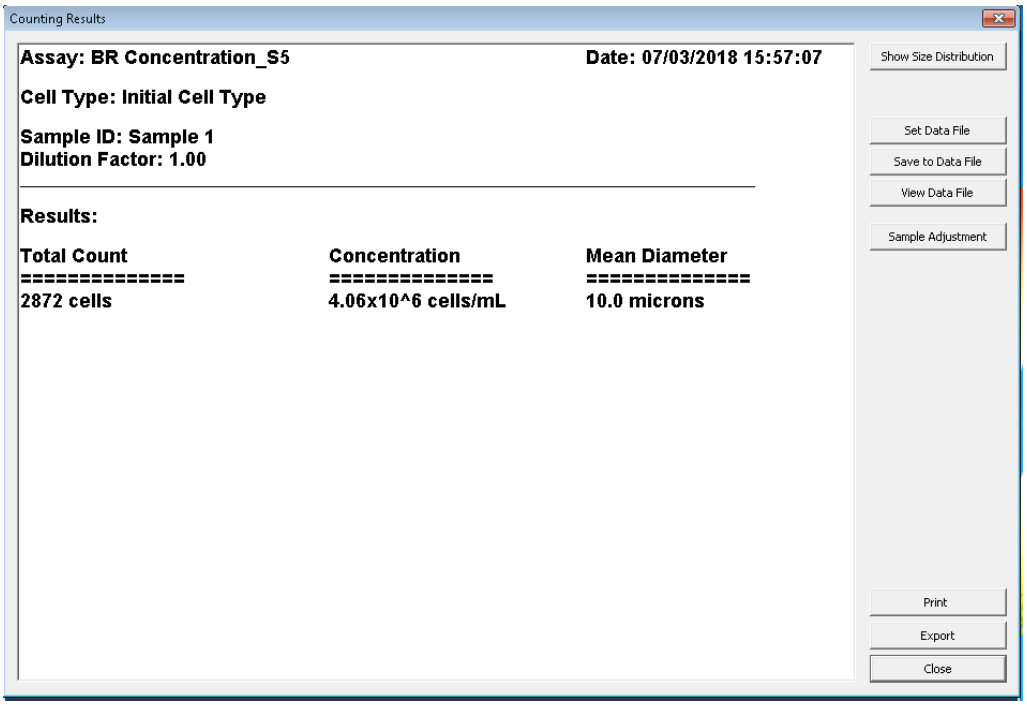
Note that Live cells and reference beads will have a bright center and a clearly defined edge.



STEP 5. CLICK 'PREVIEW F1 IMAGE'.

STEP 6. CLICK THE COUNT (OR SPEED COUNT) TO BEGIN THE COUNTING PROCESS.

STEP 7. THE COUNTING RESULTS BOX WILL DISPLAY UPON CONCLUSION OF THE COUNTING PROCESS



Initial set up and configuration are now complete. You are ready to start using Cellometer Spectrum. The next chapter contains tutorials of common applications to help familiarize you with the instrument and its functions.

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Chapter 4. Tutorial Overview

The following tutorials are intended as a guide to perform various cell-based assays using the Spectrum. General sample preparation hints are included for each tutorial, as well as instrument and software operation instructions.

Each of the assays can also be performed using the sample images included in the software as a demonstration of instrument and software operation. Sample images for each assay can be found at:

C:\Program Files\Nexcelom_Spectrum\Assay_Images

RECOMMENDED STAINING CONCENTRATIONS

The following are recommended staining concentrations when using the Cellometer:

- **Trypan Blue Staining –** Final trypan concentration with cells should be 0.1%. Recommend stock solution of 0.2% (0.2mm filtered) and mixing 1:1 with cell sample.
- **Acridine Orange (AO) –** Final concentration with cells should be 0.5µg/mL.* Recommend stock solution of 1µg/mL and mixing 1:1 with cell sample.
- **Propidium Iodide (PI) –** Final concentration with cells should be 10µg/mL.* Recommend stock solution of 20µg/mL and mixing 1:1 with cell sample.
- **Ethidium Bromide (EB)** – Final concentration with cells should be 10µg/mL.* Recommend stock solution of 20µg/mL and mixing 1:1 with cell sample.

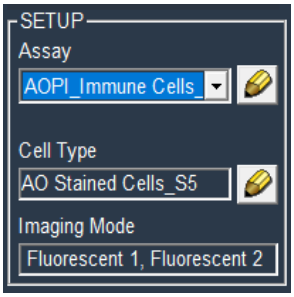
* Concentrations should be optimized for specific applications.

SAMPLE TUTORIALS

Modify Default Assay Parameters

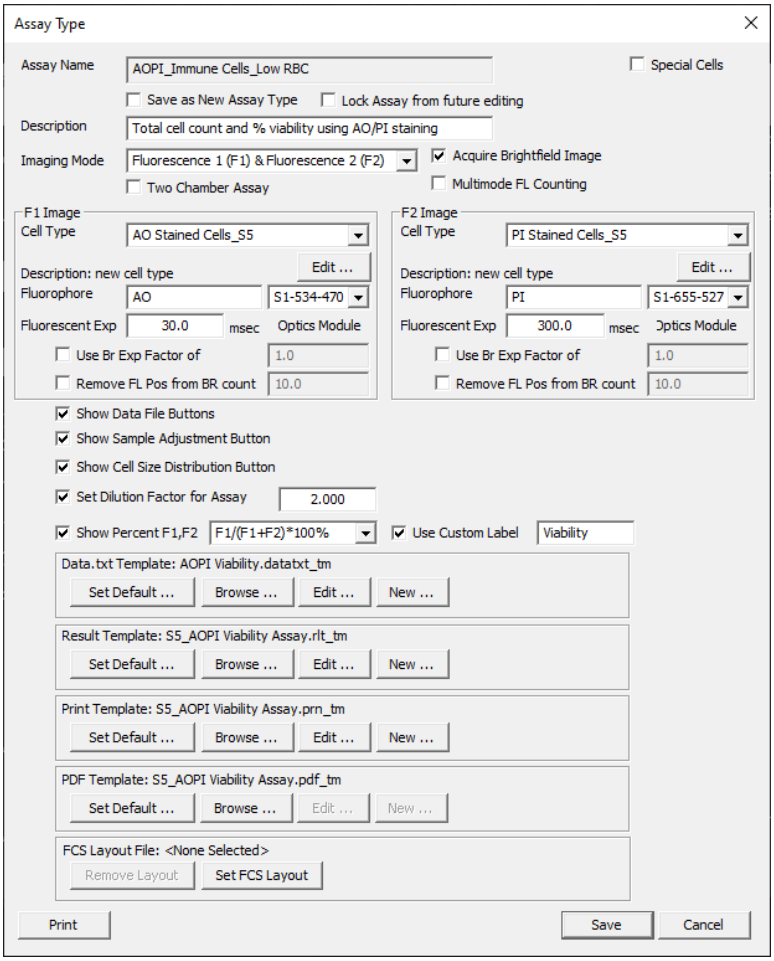
Spectrum default assay types are intended as guidelines for setting up new assays. The following is a procedure to modify an existing assay type to suit specific experiments.

STEP 1. SELECT AN ASSAY FROM ASSAY SETUP DROP-DOWN MENU



STEP 2. EDIT ASSAY TYPE BY CLICKING ON THE YELLOW PENCIL TOOL ICON

A. Click **Save as New Assay Type**.

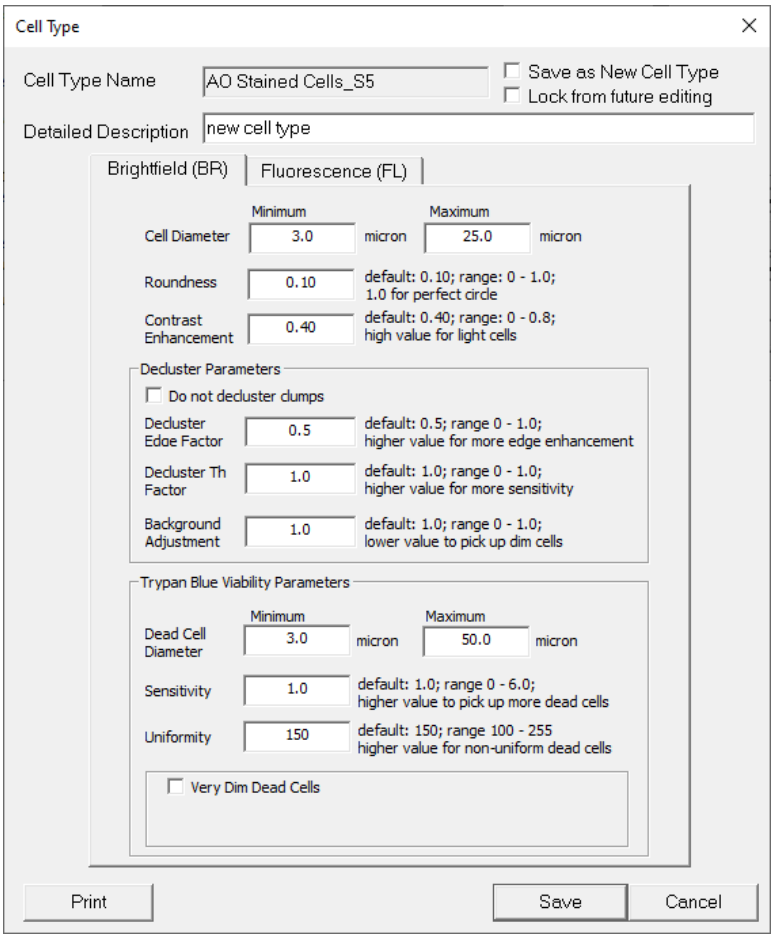


B. Input new or modified name in **Assay Name**.

C. Modify cell type if necessary.

- Method 1: Drop-down from F1 Image Cell Type to find another cell type.
- Method 2. Edit to change current cell type parameters.

Note: The **Save** button will become active once the Assay Name has changed.



D. Click **Save as New Cell Type**.

E. Change **Cell Type Name**.

F. Input modification to the cell type parameters.

Note: The **Save** button will become active once the Cell Type Name has changed.

G. Change Fluorescence exposure time if needed.

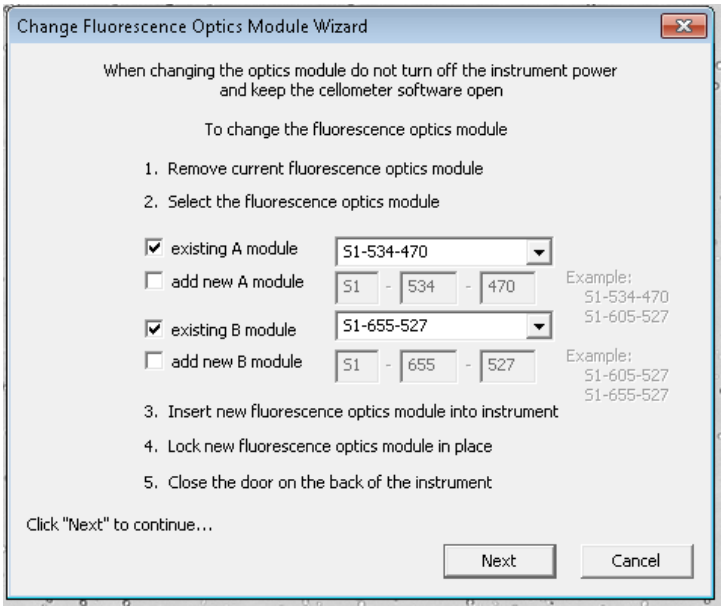
Change Optical Module

1. Preparation

a. Turn on power to Spectrum instrument (verify white LED light is on in front).
b. Connect Spectrum USB cable to the computer controller.
c. Start the Spectrum software (v3.0.0 or higher).

2. Changing Fluorescence Optics Module

a. In the Spectrum software, select: Options -> Change Fluorescence Optics Modules



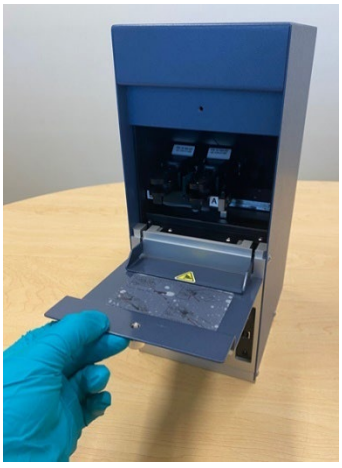
b. Rotate Spectrum instrument around, with the back side facing forward.

c. Open the Fluorescence Optics Module access door.

d. Remove shipping bracket (keep for later use for shipping Spectrum instrument if needed).

e. Identify Fluorescence Optics Module(s) to be removed and its location (A, B, or both).

f. Remove the desired Fluorescence Optics Module.



g. In the Spectrum software (Change Fluorescence Optics Module Wizard), enter the new optics module number (M/N).

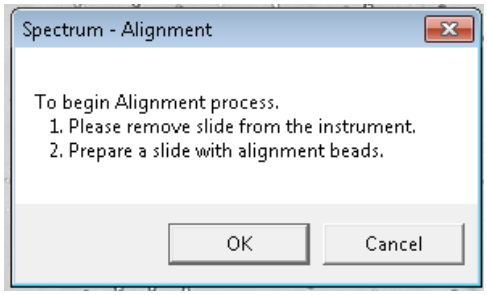
h. Insert the new Fluorescence Optics Module into the instrument.

i. Close Fluorescence Optics Module access door (wait for instrument adjustment ~1 min).

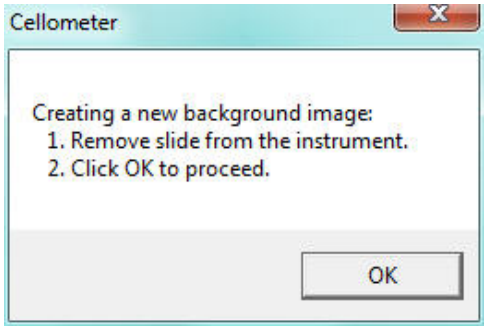
j. Rotate Spectrum instrument around with front side facing forward.

k. In Optical Module Change Wizard, click **Next**.

3. Alignment and Background Adjustment (will begin automatically after clicking 'Next' in step k. above)

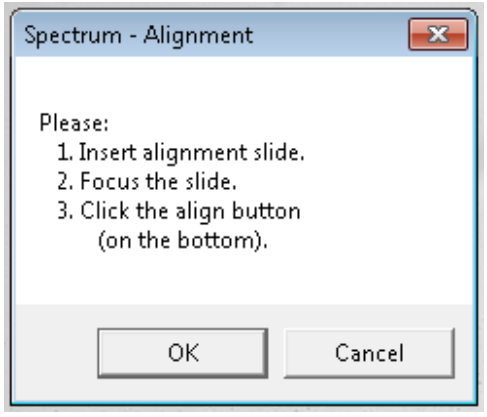


a. Without a slide in the Spectrum instrument, click **OK** for background adjustment (~2 min).



b. Prepare CCBM-011-2ML beads and load (20 µL) in a Cellometer counting chamber.

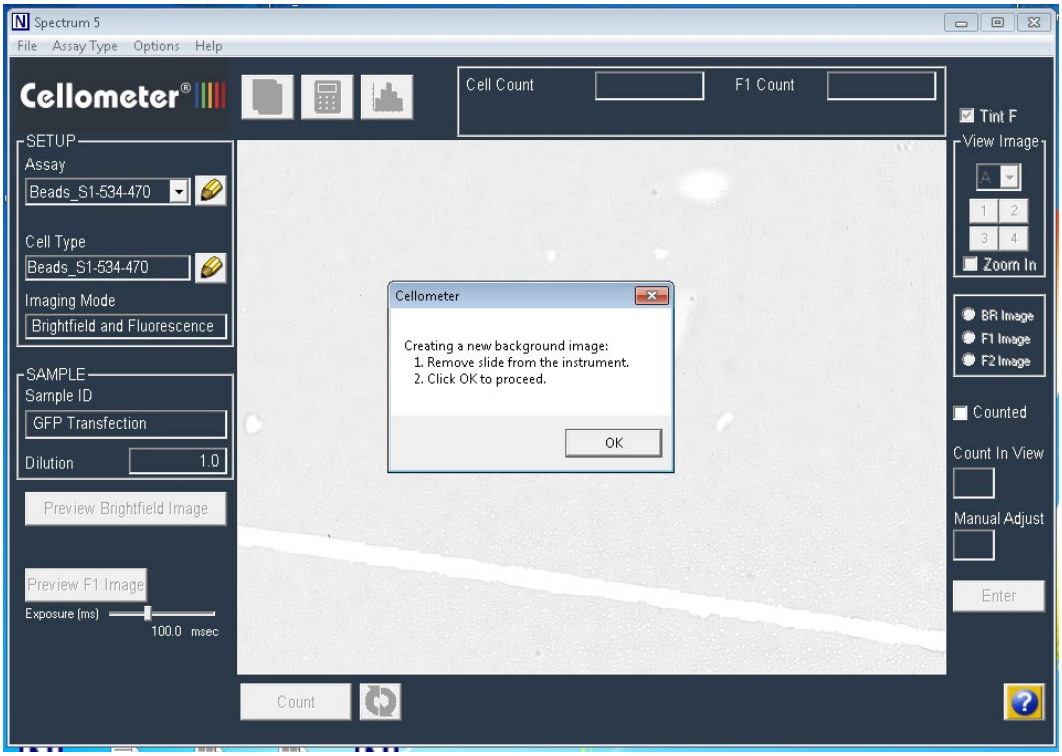
c. Insert slide loaded with CCBM-011-2ML beads into the Spectrum instrument and click **OK**.



d. Focus on the beads.

e. Click **Align Fluorescence Module** (~2 min).

f. Another set of background images will be taken after alignment.



g. New alignment and background have been established for the desired Fluorescence Optics Modules pair.

4. Verify Optical Performance

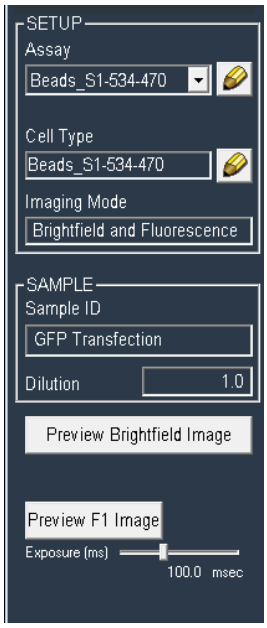
a. Prepare required CCBM-011-2ML beads into Cellometer counting chamber for the new Fluorescence Optics Modules in the Spectrum instrument.

b. Insert slide with beads.

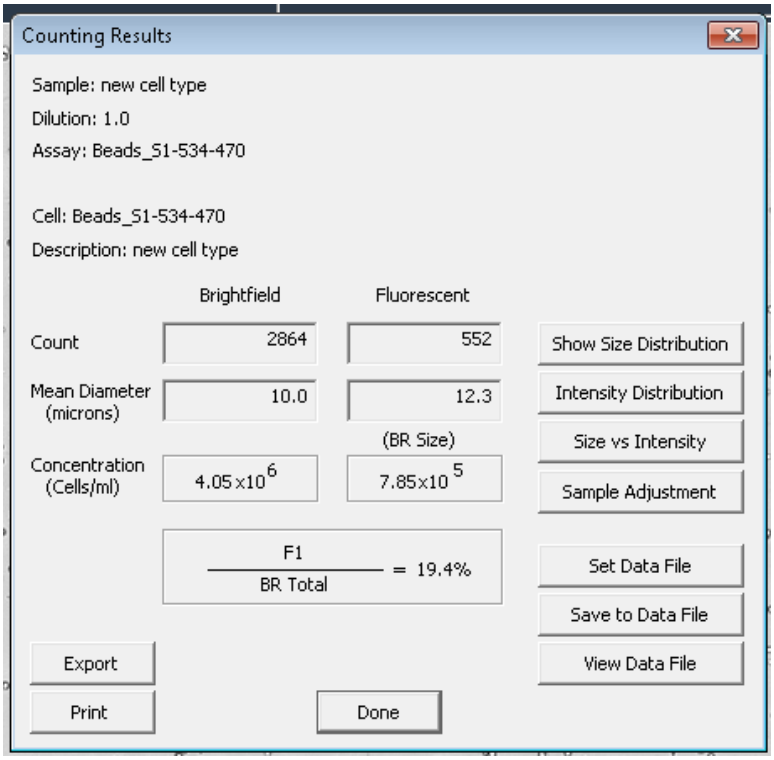
c. Select the appropriate assay type for beads verification.

d. Preview image (focus if needed).

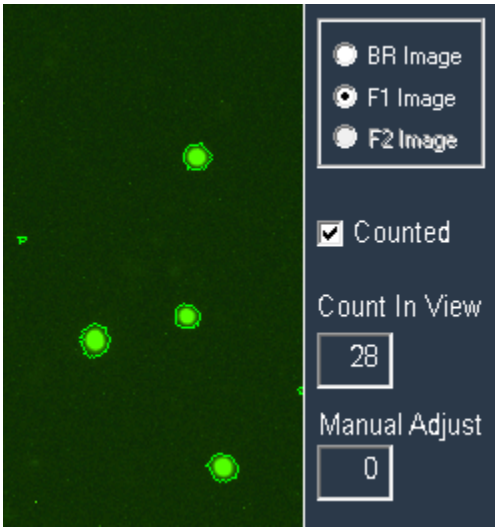
e. Click **Count**.



f. Check counted (if > 95% counted -> Verified).



g. If beads fluorescent image is not detected, check to see if the CCBM-011-2ML beads and the Fluorescence Optics Modules are selected correctly.



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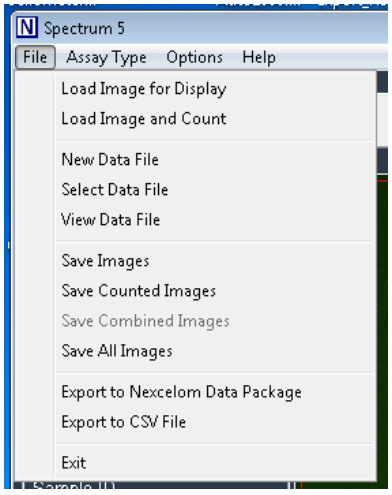
Chapter 5. Operation Reference

This chapter provides an operation reference for available software functionality menus.

FILE MENU



File > Load Image for Display

Load saved cell images for display. When Assay Type includes both brightfield and fluorescence, multiple images are required.

File > Load Image and Count

Load saved cell images for counting. When Assay Type includes both brightfield and fluorescence, multiple images are required. Saved cell images are counted using current Assay Type parameters.

Fila > New Data File

Start a new text file for Cell Count Table.

File > Select Data File

Select a different text file for saving Cell Count Table.

File > View Data File

Display currently selected Cell Count Table.

File > Save Images

Save cell images.

File > Save Counted Images

Save cell images with outlines indicating counted objects.

File > Save Combined Images

Save the combined view showing F1 and F2 fluorescent objects in a single merged image (available only when an assay uses both F1 and F2 images).

File > Save All Images

Save all cell images captured during the count.

File > Export to Revvity Data Package

Export cell images as .NXDat file which can be opened with FCS Express® software.

File > Export to CSV File

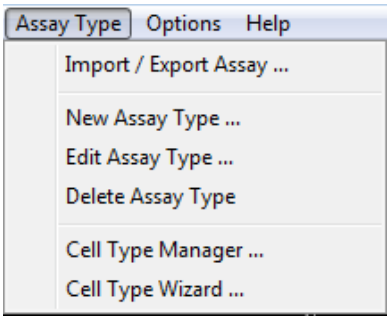
Export cell images to MS Excel .csv file.

File > Exit

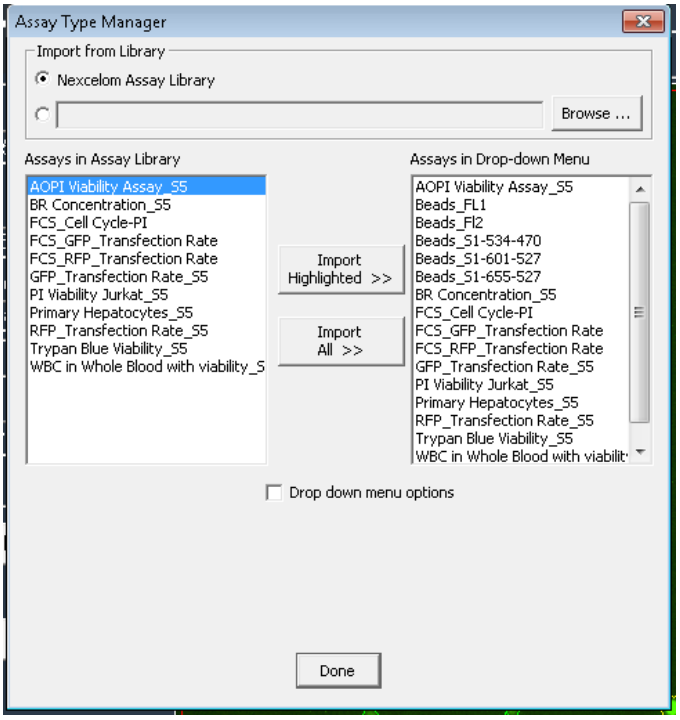
Quit Cellometer Spectrum software.

ASSAY TYPE MENU

Assay Type > Import/Export Assay



The Assay Type Library is a list of defined assay types. Assay Type Manager is used to import from the existing assay library or to export a user-defined list of assay types into a new Assay Library.



Nexcelom Assay Library: Default built-in Assay Library included with the Cellometer software.

Browse: Select location from which to load a saved Assay Library.

Assay in Assay Library: Lists all Assay Types available in the selected Assay Library.

Assays in Drop-down Menu: Lists assays to be displayed in the Spectrum software Setup Assay Drop-down list.

Import Highlighted Button: Import highlighted assay types from selected Assay Library (displayed on the left) to Assays in Drop-Down Menu (displayed on the right).

Import All Button: Import complete list of assays from selected Assay Library (displayed on the left) to the Assays in Drop-Down Menu (displayed on the right).

Drop-down Menu Options Check Box: De-select to hide delete and export buttons:

Delete Highlighted: Delete highlighted assay type.

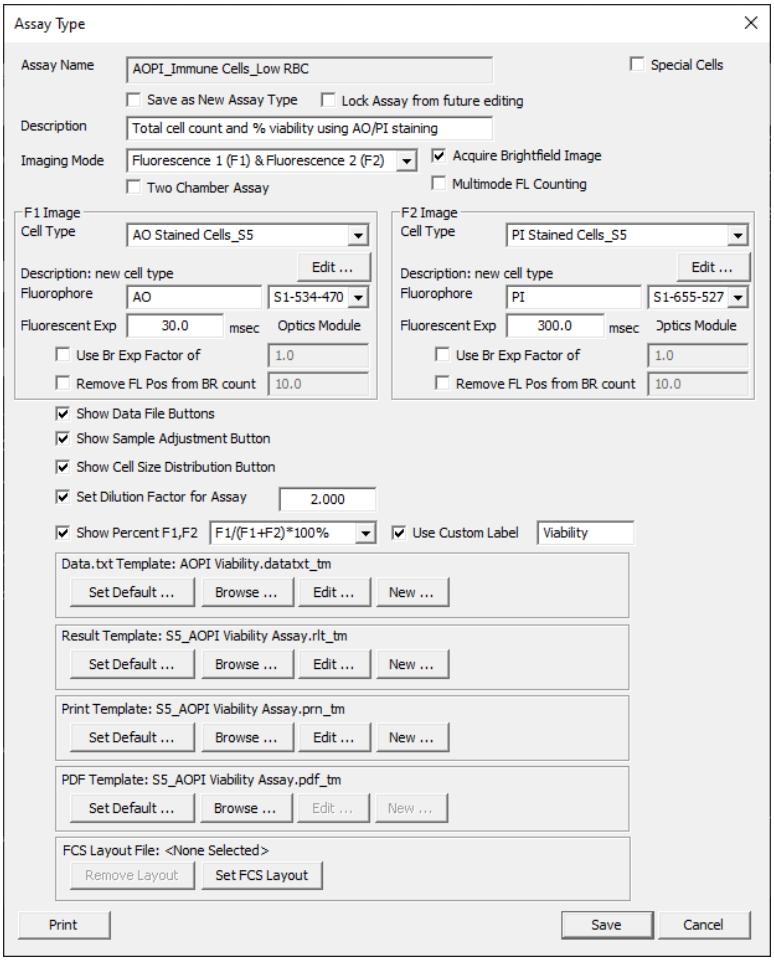
Clear All: Clear all assay types in the drop-down menu.

Export to Create Library: Export all assay types in the drop-down menu to a new Assay Library.

Export Highlighted: Export only highlighted assay types in the drop-down menu to a new Assay Library.

Done: Close Assay Manager window.

Assay Type -> Edit Assay Type



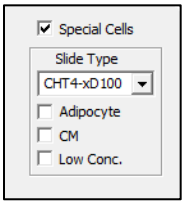
Assay Name: Name of assay that appears in the Assay drop-down list.

Save as New Assay Type: Check to save current Assay Type with a different name. User can then edit parameters for new Assay Type (See *Modify Default Assay Parameters* on page *22* for details).

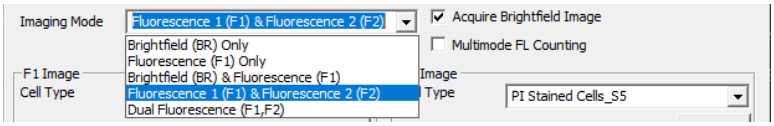
Lock Assay from Future Editing: Check to prevent future editing. Parameters cannot be changed later. Default assays are locked in software upon installation.

Description: Text descriptions for your assay.

Special Cells Check Box: Check here to use specialized counting algorithms and to change the counting chamber slide type.



Imaging Mode:



Brightfield (BR) Only: Use only brightfield to count cells and measure size.

Fluorescence (F1) Only: Use only fluorescence to count cells.

Brightfield (BR) & Fluorescence (F1): Acquire both brightfield and fluorescence images. Cell counting results are produced using a combination of both images.

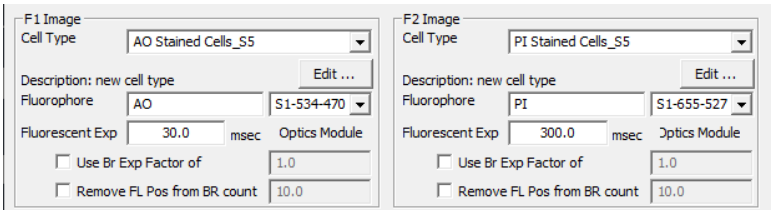
Fluorescence 1 (F1) & Fluorescence 2 (F2): Use two different fluorescence images to produce counting results.

Dual Fluorescence (F1, F2): Identifies cells that show fluorescence in both F1 and F2 images.

Note: Fields displayed may vary depending on selected *Imaging Mode*.

Acquire Brightfield Image: Acquire brightfield image when a copy is needed for visual verification but not for performing count (e.g., Imaging Mode set to FL only: Analysis is performed for cell counts using FL images. Brightfield image is captured only for visual verification that cell sample is good).

Multimode FL Counting: Measure fluorescence intensity only within cells located in Brightfield (BR) image (available only in Fluorescence counting modes).



F1 Image: Contains Cell Type and Fluorescence parameters for F1 image.

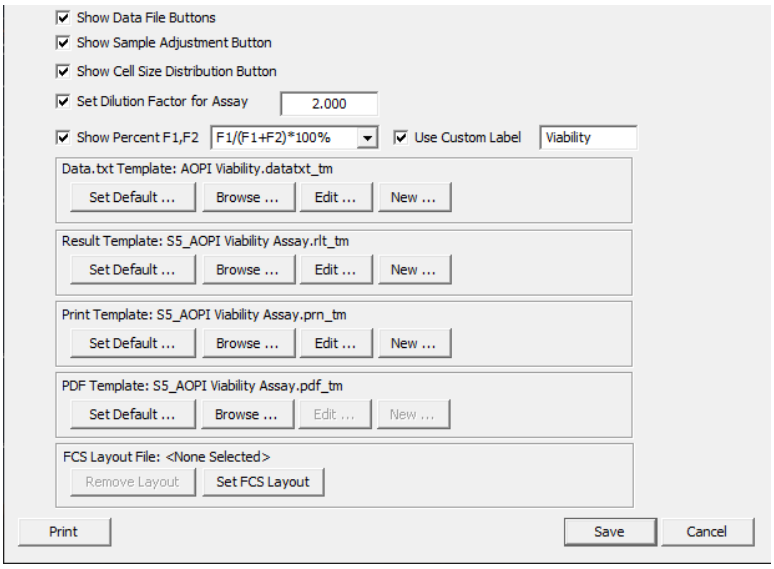
F2 Image: *Not shown; visible in 'Fluorescence 1 and Fluorescence 2' Imaging Mode.* Contains Cell Type and Fluorescence parameters for F2 image.

Cell Type: Drop-down menu to select cell parameters.

Edit: Edit selected cell type parameters.

Fluorophore: User-defined name of Fluorophore used in this assay (Note: VA-535-401 or VA-595-501 indicates the filter set that detects that specific fluorophore).

Fluorescent Exp: Fluorescence exposure time in milliseconds.



The following options allow you to customize which results are displayed in the Counting Results box:

Show Data File Buttons: Check to show the 'Set Data File', 'Save to Data File' and 'View Data File' Buttons in report window.

Show Sample Adjustment Button: Check to show the 'Sample Adjustment' button in report window.

Show Cell Size Distribution Button: Check to show the 'Show Size Distribution' button in report window.

Set Dilution Factor for Assay: Enter value used as the dilution factor for the original cell concentration calculation.

Show Percent F1,F2: Indicates how result is calculated. For example:

- To show percentage of F1 Positive Cells within total fluorescent positive cells, choose: F1/(F1+F2)*100%
- To show percentage of F2 Positive Cells within total fluorescent positive cells, choose: F2/(F1+F2)*100%

Note: Additional calculations will appear for other Imaging Modes.

Use Custom Label: Allows user to create a customized label for the calculated percentage in report window.

Print: Print out selected Assay Type parameters.

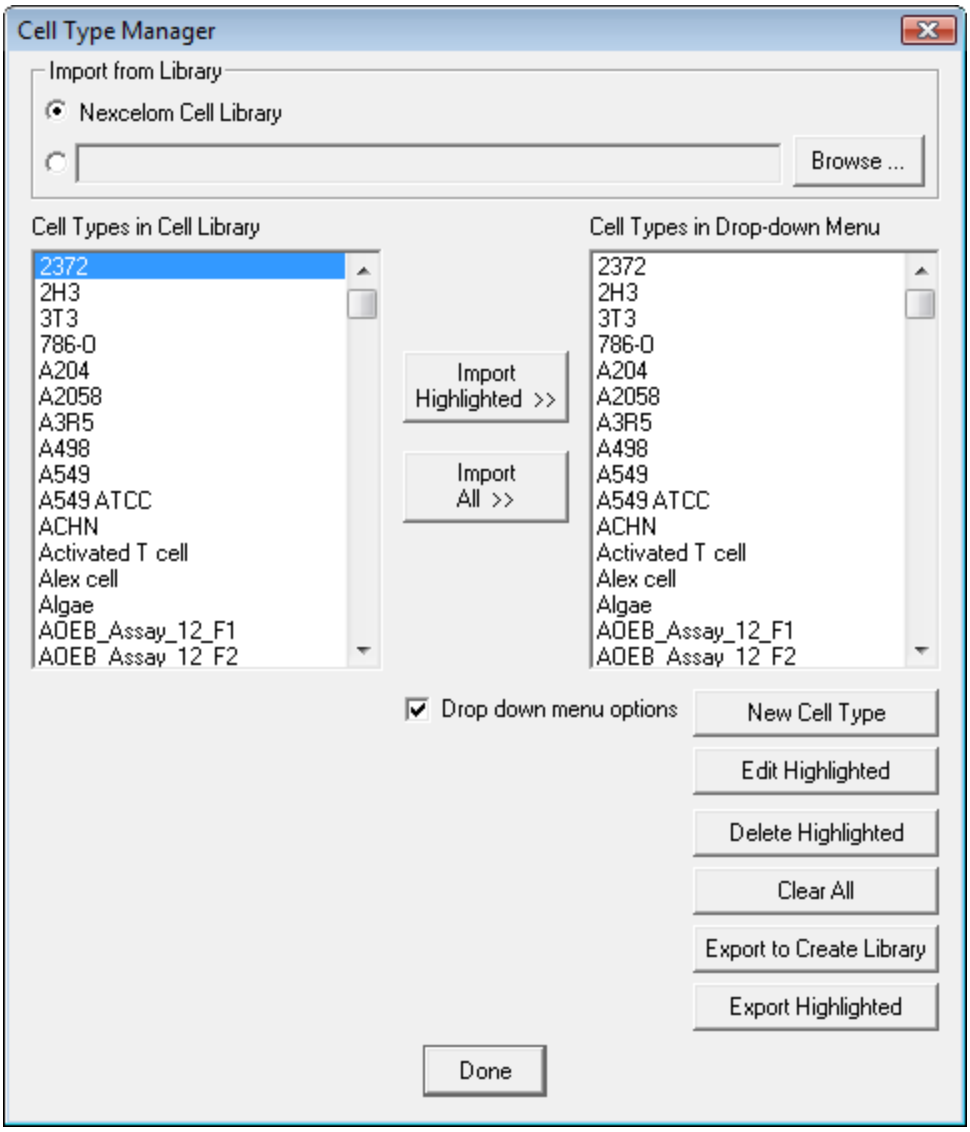
Save: Saves changes.

Cancel: Close Assay Type without saving changes.

Assay Type > Delete Assay Type

Delete selected assay from Setup Assay drop-down list.

Assay Type > Cell Type Manager



Nexcelom Cell Library: Default built-in Cell Library included with the Cellometer software.

Browse: Load a user-defined Cell library.

Cell Types in Cell Library: List of Cell Types in the current Cell Library.

Cell Types in Drop-down Menu: List of Cell Types in the drop-down menu located in the Assay Type editor.

Import Highlighted >>: Import highlighted cell type from a Cell Library (displayed on the left).

Import All >>: Import all cell types from the Cell Library (displayed on the left).

Drop-down Menu Options: Check to see details.

New Cell Type: Start a new cell type from default setting.

Edit Highlighted: Edit the highlighted cell type.

Delete Highlighted: Delete highlighted cell type.

Clear All: Clear all cell types in the drop-down list.

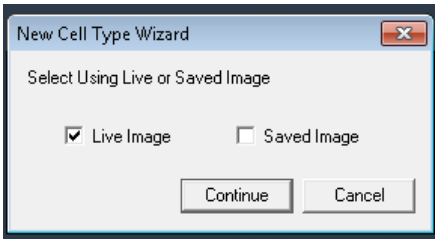
Export to Create Library: Export all the cell types in the drop-down menu to a new Cell Type Library.

Export Highlighted: Export only the highlighted cell types in the drop-down menu to a new Cell Type Library.

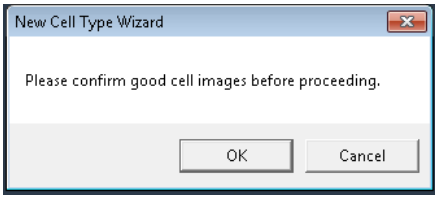
Done: Closes Cell Type Manager window.

Assay Type > Cell Type Wizard

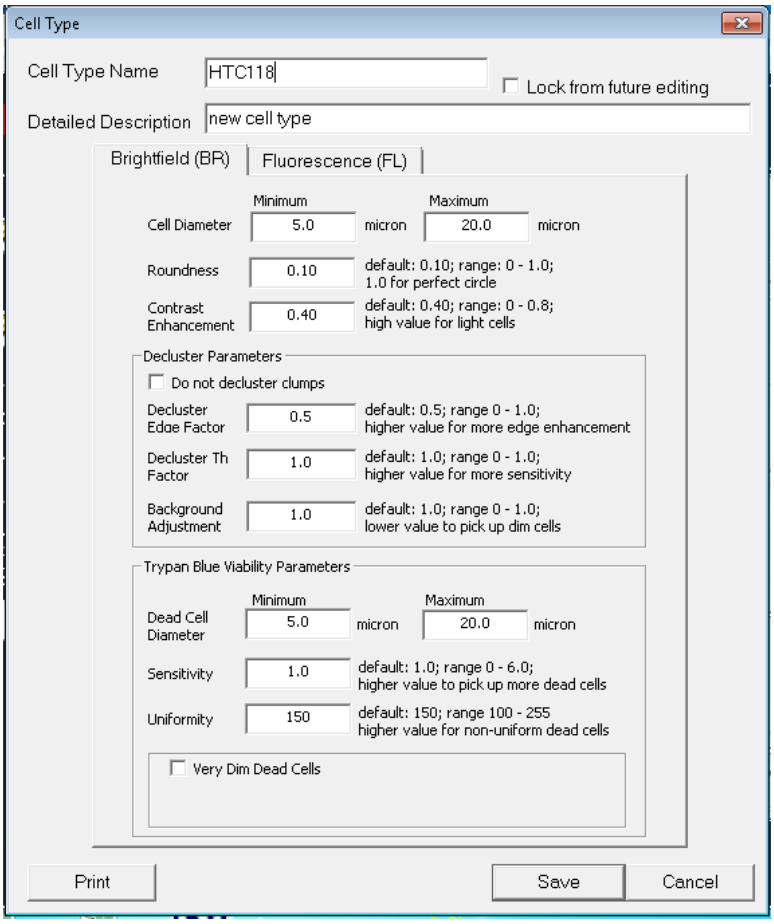
Select type of cell sample (*Live Image* for cells in CHT4 or *Saved Image* for a .bmp file of a saved cell image).



For a *Live Image*, confirm that cells are in the CHT4 counting chamber and inserted into the instrument.



Cell Type Wizard processes images and calculates Cell Type Parameters to be used. User has ability to give entered parameters a name so they can be saved for future counts.



Cell Diameter Minimum: Value indicates minimum diameter of a cell that will be included in the count.

Cell Diameter Maximum: Value indicates maximum diameter of a cell that will be included in the count.

Roundness: Value indicates which shape of cells to be included in the count.

Suggested value: 0.10; Range 0.10 to 1.00
0.10 (All shape of cells included in count)
1.00 (only perfectly round cells counted)

Contrast Enhancement: Value indicates what imaging enhancement is needed for cells to be identified.

Suggested value: 0.4; Range 0.01 to 0.90
0.01(Cells have high contrast to background)
0.90 (Cells have low contrast to background)

Do not decluster clumps: Check if desired not to count individual cells within a clump. Clump of cells will be counted as one cell if size of clump falls within cell min and max range.

Decluster Edge Factor: Value indicates the degree in which the software needs to enhance the cell edge for declustering.

Suggested value: 0.5; Range 0.00 to 1.00
0.0 (Cells have well defined edges)
1.0 (Cells do not have well defined edges)

Decluster Th Factor: Value indicates the threshold ratio between the cell signal and the background.

Suggested value: 1.0; Range 0.00 to 1.00
0.0 (Cell signal to background is very low)
1.0 (Cell signal to background is high)

Background Adjustment: Value indicates threshold adjustment needed to pick up strong or weak cells.

Suggested value: 1.0; Range 0.00 to 1.00
0.0 (Cells are very dim)
1.0 (Cells are well defined)

Dead Cell Diameter Minimum: Value indicates minimum diameter of a trypan blue stained cell that will be included in the count.

Dead Cell Diameter Maximum: Value indicates maximum diameter of a trypan blue stained cell that will be included in the count.

Sensitivity: Value adjusts the range of trypan blue stained cells that will be included in the count.

Suggested value: 1.0; Range 0.10 to 5.00
0.10 (Cells are stained DARK with trypan blue)
5.00 (Mix of cells stained DARK and LIGHT with trypan blue)

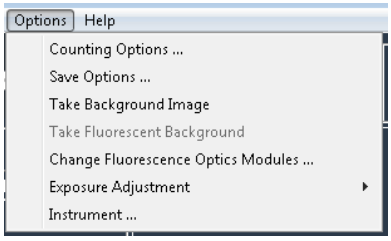
Uniformity: Value indicates if the trypan blue stained cells are uniformly stained or if there are cells with light and dark areas of staining.

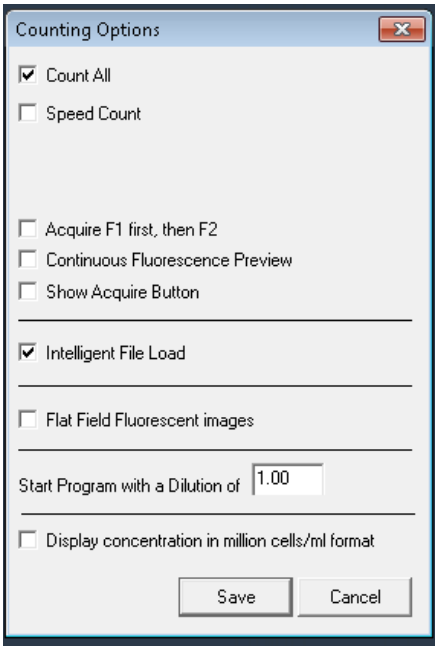
Suggested value: 150; Range 100 to 255
100 (Cell uniformly stained with trypan blue)
255 (Cell has dark and light areas stained with trypan blue)

Very Dim Dead Cells: Checking this box indicates the software uses a special function enhancement to locate and count dead cells that have weak contrast.

OPTIONS MENU

Options > Counting Options





Count All: Check to count all four pre-defined locations inside each counting chamber.

Speed Count: Check for count without finishing all four pre-defined locations.

Stop after # of Cells: Check to stop counting after # of user defined cells are counted and the next frame has finished counting.

Stop after # of Images: Stops counting after finishing user-defined images that are less than 4 images.

Acquire F1 first, then F2: Sets the fluorescence imaging capture method to acquire F1 images first then F2 images. If not checked the method alternates between F1 and F2 images for locations A, B, C and D.

Continuous Fluorescence Preview: Allows user to adjust focus in Preview F1 Image mode.

Show Acquire Button: Allows user to acquire the set of fluorescence and brightfield images for review without having to perform a count.

Intelligent File Load: Allows user to select one .bmp file image for loading and the software will identify and load all additional images related to that image.

Flat Field Fluorescent Images: Allows fluorescent background image to be used for data analysis (FL reference slides needed).

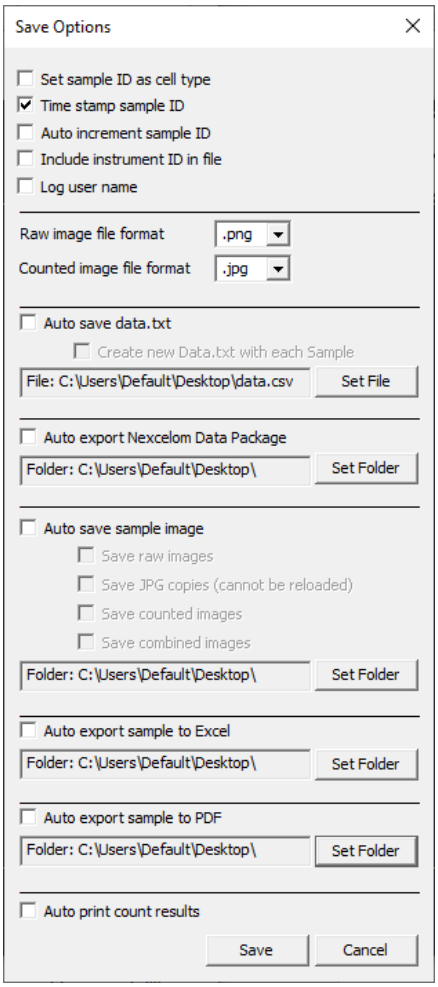
Start Program with a Dilution of: Allows user to set a default dilution factor for when software starts.

Display concentration in million cells/ml format: Sets results to be displayed in x million cells/mL in report window.

Save: Closes Counting Options dialog box and saves changes.

Cancel: Closes Counting Options dialog box without saving changes.

Options > Save Options



Set Sample ID as Cell Type: Checking this box will auto input the Sample ID to match the Cell Type parameter name being used for counting.

Time Stamp Sample ID: Checking this box will auto append the Sample ID with the date and time the count was performed.

Auto Increment Sample ID: Checking this box will auto append the Sample ID with an incremental numerical value (Example: CHO sample_001).

Include Instrument ID in File: Checking this box will auto append the Instrument ID to the sample ID after the count is performed.

Log User Name: Checking this box requires the user to enter in an ID that will be recorded with the data.

Image File Save Options:

- User has two options for file formats to save raw images (.bmp and .png). Raw images can be used to Load Image and Count. *Note that .png files are 1/3 the file size of .bmp files.*
- User has three options for file formats to save counted images (.bmp, .png and .jpg). Counted images display outlines of cells counted and cannot be used to Load Image and Count. *Note that .jpg files are 1/20 of the file size of .bmp files.*
- To change file format, go to: **Options > Save Options …**
- Select the desired file formats.
- Click **Save**.

Auto Save data.txt: Checking this box auto saves the data into the **Data.txt** file after a count is performed.

Create new Data.txt with each Sample: Checking this box will auto create a new data.txt file for each sample and save the counting results to it after a count is performed.

Auto Save Sample Image: Checking this box will auto save the images to the folder specified using the Set Folder command.

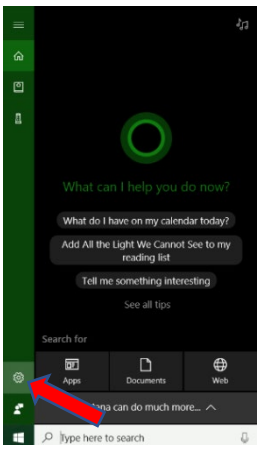
Save Raw Images/Save Counted Images/Save Combined Images: Checking these boxes indicate which type of image will be saved when the Auto save sample image box is checked.

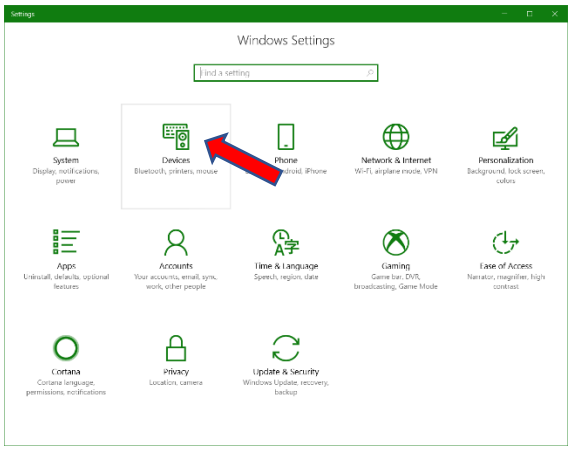
Auto Export Sample to Excel: Checking this box auto exports the counting data, results and calculations into an Excel file which will be saved in the location specified by using the Set Folder command.

Set Folder: These buttons bring up the File Dialog Window which allows the user to specify where the data.txt, images and/or Excel files will be saved when Auto Save boxes are checked.

Auto Print Count Results: Click **Save** button.

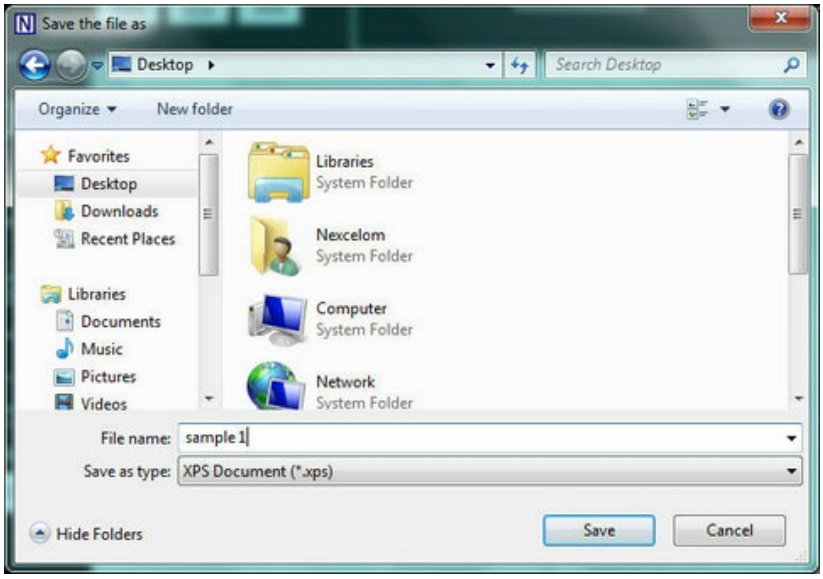
- **Note #1:** Only works with Live Image, not for Load Image and Count.
- **Note #2:** Set up a printer before using Auto-printing from Devices and Printers.



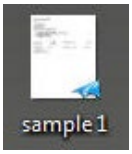


If not connected to a printer, the print dialog will automatically select the default system printer. For Windows 10 Operating system, *.xml is the default printer.

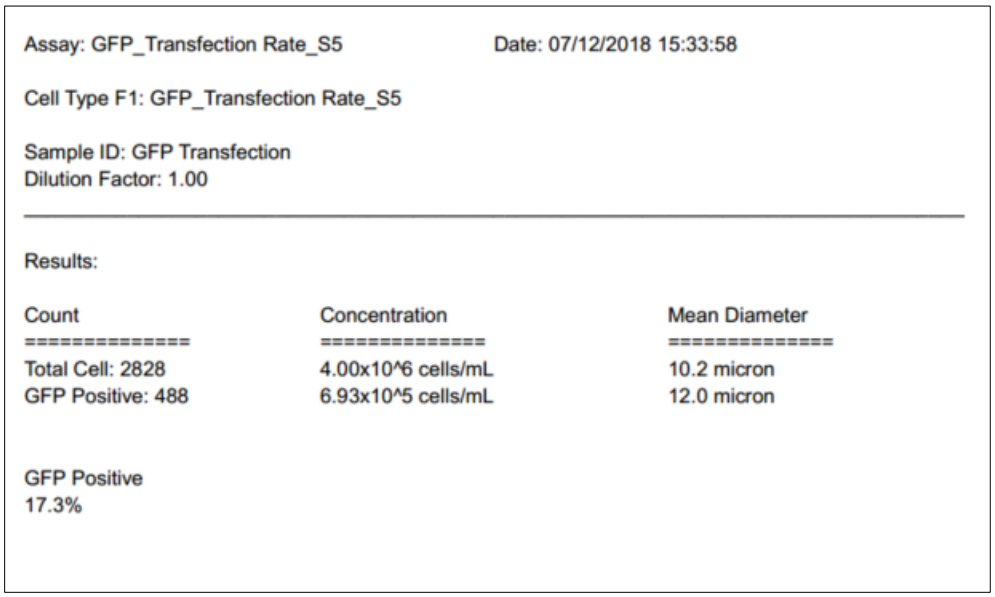
When counting is complete and Microsoft XPS is default printer, a dialog box will appear. Give file name and location to save.



To view printed XPS document, double click on saved file.



Note #3: Auto-printing uses default count result template. If you need to customize the report, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com



Assay: GFP_Transfection Rate_S5 Date: 07/12/2018 15:33:58

Cell Type F1: GFP_Transfection Rate_S5

Sample ID: GFP Transfection
Dilution Factor: 1.00

Results:

Count	Concentration	Mean Diameter
Total Cell: 2828	4.00x10^6 cells/mL	10.2 micron
GFP Positive: 488	6.93x10^5 cells/mL	12.0 micron

GFP Positive
17.3%

Options > Take Background Image

Takes brightfield background image of the Spectrum system without cell counting chamber. For Spectrum Trio instruments, Background Images are needed for both Fluorescence Optics Modules.

Brightfield exposure times will automatically be set and saved.

Options > Take Fluorescent Background

Takes a fluorescence background image to be used for data analysis (FL reference slices needed).

Options > Change Fluorescence Optics Modules

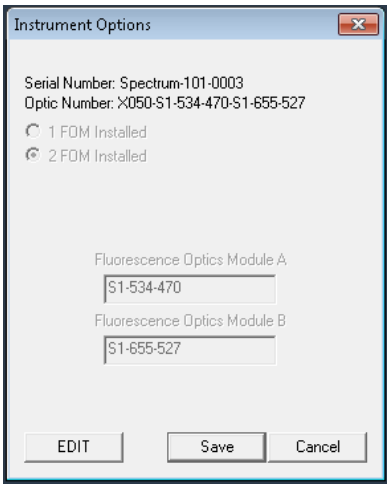
Allows you to change fluorescence optics modules as necessary. See *Change Optical Module* on page *24*.

Options > Exposure Adjustment

Show Exposure Adjustment: Display Exposure time.

Save Exposure Time as Default: Save an Exposure time that has been changed as default. When the Spectrum software is closed and restarted, the saved exposure time will be used.

Options -> Instrument



Note that these are factory settings and are only editable when changing hardware configuration. For additional information, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

Instrument: Indicates the name of the instrument assigned during installation.

Serial Number: Indicates serial number of instrument (found on back of hardware).

Optics Number: Indicates instrument magnification settings and fluorescence optical modules installed.

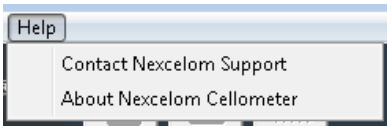
Spectrum Duo/Spectrum Trio: Indicates instrument for which the software is configured (Factory Setting).

Fluorescence Optics Module A/B: Displays the current fluorescence optics modules installed and location.

HELP MENU

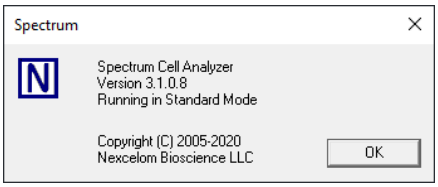
Help>Contact Nexcelom Support

Opens Cellometer Spectrum Support web page.



Help> About Nexcelom Cellometer

Displays version number of Cellometer Spectrum software currently installed.



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Chapter 6. Technical Information

This chapter contains instrument specifications, environmental requirements for intended operation, troubleshooting recommendations, and available online Support resources.

INSTRUMENT SPECIFICATIONS

Instrument Specifications

- Weight: 24 lbs (10.9 kg)
- Dimensions:

  Width: 6.0” (15.2 cm)
  Depth: 8.5” (21.6 cm)
  Height: 14” (35.6 cm)
- Input to Power Adaptor: 100-240 AC, 50-60 Hz, 1.0 A
- Output to instrument: 12 V DC, 3.34 A

Declaration of Conformity

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

ENVIRONMENTAL REQUIREMENTS

Environmental requirements for the intended operation of Cellometer Spectrum instruments 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.

TROUBLESHOOTING

If the software displays an error or hangs during image preview/acquisition, exit the application by closing the window and re-launch the Cellometer software. If the error persists, power cycle the instrument and then restart the Operating Computer. Launch the software and proceed with image preview/acquisition.

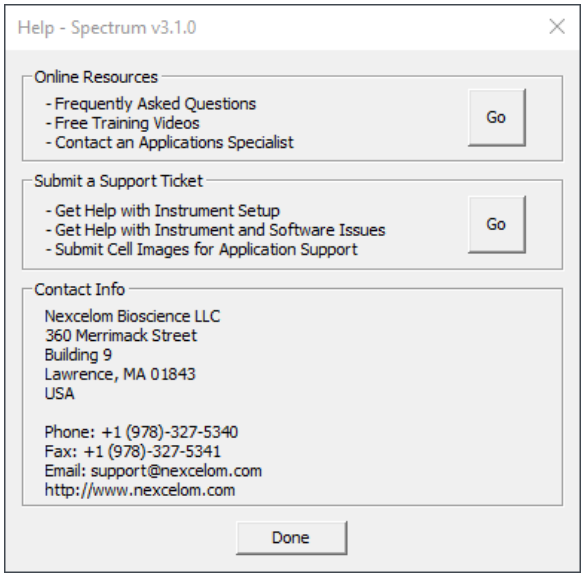
In cases where this issue is *not* resolved by the procedure above or if it continues to happen on a regular basis, contact Support by visiting https://www.revvity.com/contact-us or send email to: CellC-support@revvity.com

ONLINE SUPPORT RESOURCES

Revvity offers online Support resources directly from the Spectrum software if you have an internet connection.

By selecting the  icon you will be offered help contents regarding getting started with your instrument, software features and operational references. You may also seek advice from frequently asked questions, view free training videos or contact Support.

From your software you can submit a support ticket with cell images attached. This will allow Support to help you with the analysis of cell images, modifying instrument settings or other support-related tasks.



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

Chapter 7. 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 a technical issue encountered cannot be resolved 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 lists Revvity consumables designed for Spectrum instruments such as disposable chambered slides, reagents/reagent kits and reference beads. Catalog numbers are provided for all available sizes.

COUNTING CHAMBER SLIDES

Revvity offers Cellometer *Counting Chamber Slides* 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 of *Cellometer Disposable Counting Chambers* or contact your Revvity Sales representative to purchase Cellometer counting chamber slides directly from Revvity.

REAGENTS AND REAGENT KITS

Revvity offers a variety of assay 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 fluorescent and brightfield *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.*

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 Revvity 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 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.

Revvity retains all ownership rights in the Revvity Proprietary Information and, other than limited license set forth in this section, Buyer shall have no right in or to the Revvity Proprietary Information.

Buyer will not disclose the Revvity Proprietary Information to any third party or use it in any manner outside the scope of the license including: (1) developing, designing, manufacturing, engineering, reverse engineering, refurbishing, selling or offering for sale items, parts or components of items, derivatives of or equivalents, or (2) assisting any third party in any manner to perform such activity.

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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