

USER MANUAL

CELLOMETER[®] X2 K2



8001563 Rev D

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

CE

Cellometer® X2 K2 User Manual

8001563 Rev D

February 2024

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Chapter 1. Introduction

This chapter describes the Cellometer X2 and K2 instruments, provides quick operation instructions and presents an overview of the software, terminology and screen elements used.

CELLOMETER X2 K2 OVERVIEW

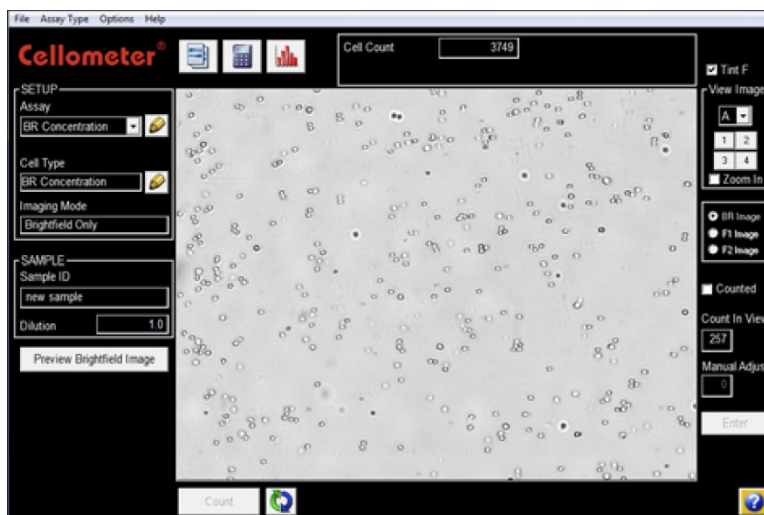
The *Cellometer X2* and *Cellometer K2* will be referred to from this point on in this user manual as just *Cellometer*.

Cellometer® 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 Cellometer automatic cell counter is *Imaging Cytometry*. Cells are loaded into the Disposable Counting Chamber and automatically spread into a thin layer by capillary action. Cellometer 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 system consists of three main components:

- Cellometer X2 | K2 instrument
- Cellometer 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 20 μ L into one of the ports with any standard single channel pipette. Cellometer comes with a starter set of 75 slides. Slides can be ordered directly from Revvity or your authorized Revvity representative. See *Disposable Chambered Slides* on page 45 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 X2 K2 instrument must meet or exceed the specifications listed above.

GENERATING DATA WITH CELLOMETER X2 K2

Generating cell sample information is quick and easy. Once the instrument is set up for your particular assay, only three basic steps are needed to get results:



1. Prepare sample and load counting chamber.



2. Insert counting chamber into Cellometer instrument.

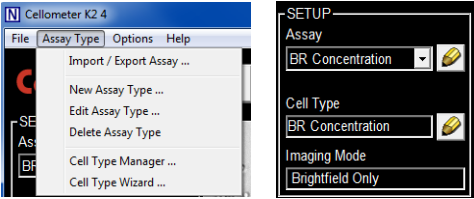
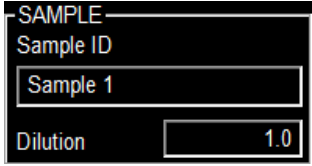

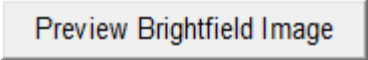

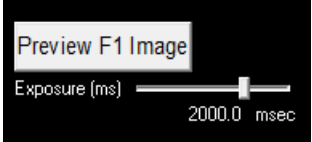
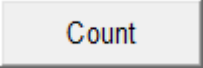
Assay: BR Concentration		Date: 09/18/2013 13:55:48	Show Test Distribution
Cell Type: BR Concentration			
Sample ID: new sample			
Dilution Factor: 1.00			

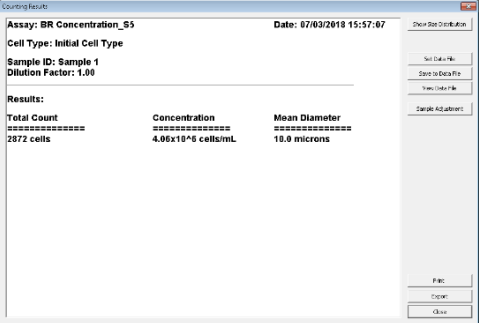


Results:			
Total Count	Concentration	Mean Diameter	
3749 cells	6.47x10 ⁻⁶ cells/mL	6.4 microns	

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

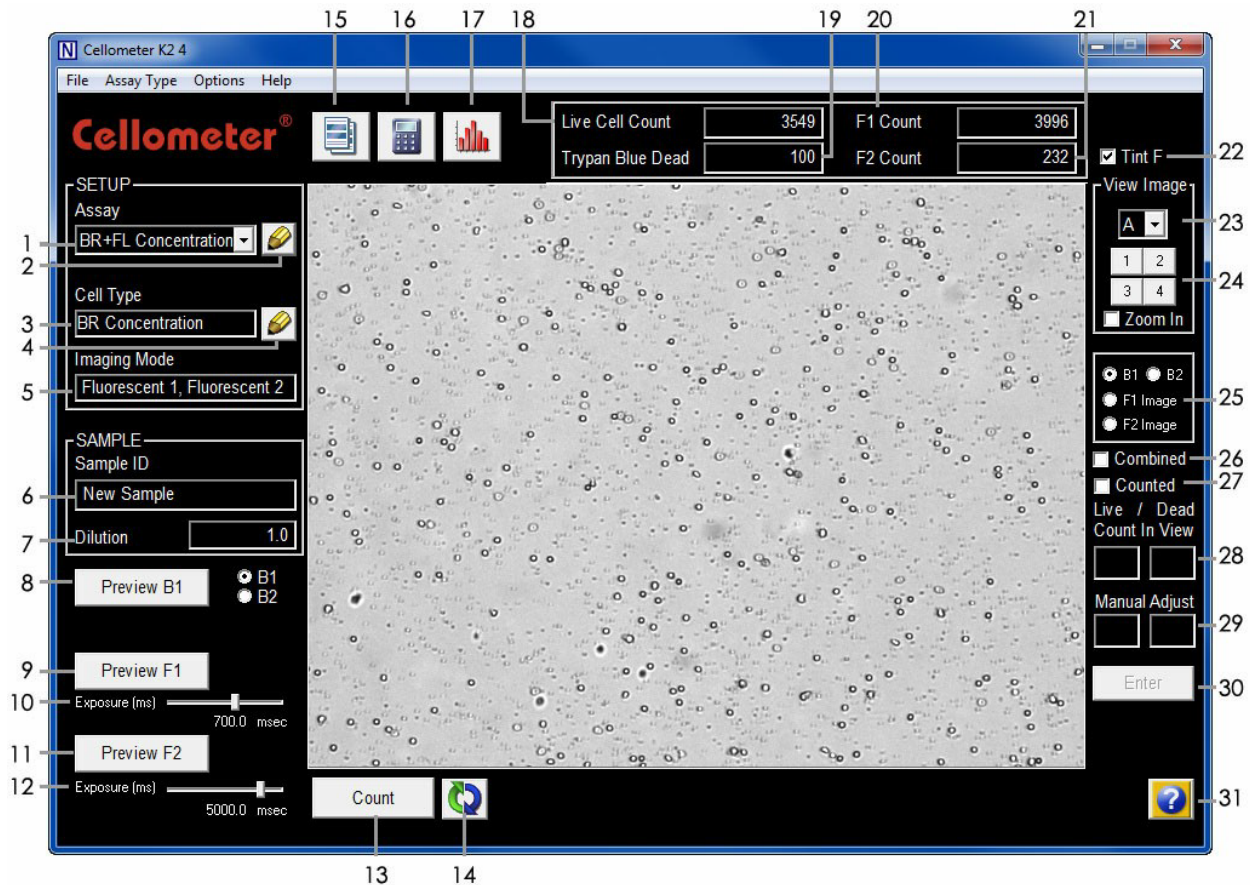
QUICK OPERATION INSTRUCTIONS

The following table presents basic steps to run any assay.

Step	Instructions	
1	<p>Set up or select an assay.</p> <p>(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.</p>	 <p style="text-align: center;">(a) and (c) (b)</p>
2	<p>Input Sample ID and Dilution Factor.</p>	
3	<p>Prepare sample, load disposable counting chamber and insert into instrument.</p>	
4	<p>Click 'Preview Brightfield Image' and ensure image is in focus.</p>	
5	<p>Adjust focus.</p> <p>Slowly turn the focus wheel until optimal cell counting focus is achieved. <i>Live cells and training beads will have bright centers and clearly defined edges.</i></p>	
6	<p>Click 'Preview F1 Image' and adjust fluorescent exposure time if necessary.</p> <p>If using dual fluorescence mode: Click 'Preview F2 Image' and adjust exposure time if necessary.</p>	
7	<p>Click 'Count' to begin counting process.</p>	

Step	Instructions	
<p>8</p>	<p>Review counting results and generate reports.</p> <p>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.</p>	 <p>The screenshot shows a window titled 'Counting Results' with the following information:</p> <ul style="list-style-type: none"> Assay: BR Concentration_85 Date: 07/03/2018 15:57:07 Cell Type: Initial Cell Type Sample ID: Sample 1 Dilution Factor: 1.00 Results: <ul style="list-style-type: none"> Total Count: 2872 cells Concentration: 4.06×10^6 cells/mL Mean Diameter: 10.0 microns
<p>9</p>	<p>Navigate and review images on screen.</p> <p>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.</p>	 <p>(a) View Image: A 2x2 grid of numbered buttons (1, 2, 3, 4) with a dropdown menu set to 'A'.</p> <p>(b) Image Selection: Radio buttons for 'BR Image', 'F1 Image', and 'F2 Image'.</p> <p>(c) Manual Adjust: A control panel with a checked 'Counted' box, 'Count In View' (262), 'Manual Adjust' (0), and an 'Enter' button.</p>
<p>10</p>	<p>If desired, click (a) <i>Re-display Counting Results</i>, (b) <i>Launch Sample Adjustment Calculator</i> or (c) <i>Display Size Distribution</i> icons.</p>	 <p>(a) Re-display Counting Results icon (document with chart).</p> <p>(b) Launch Sample Adjustment Calculator icon (calculator).</p> <p>(c) Display Size Distribution icon (bar chart).</p>

SOFTWARE OVERVIEW



Item:	Name:	What it does:
1	Select Assay	Selects a saved assay from the Assay Library.
2	Modify Assay	Modifies parameters of currently selected Assay.
3	Cell Type Display	Displays the selected cell type.
4	Modify Cell Type	Modifies parameters of currently selected Cell Type.
5	Imaging Mode Display	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.
9	Preview F1 Image	Previews the fluorescent image: F1
10	F1 Exposure Adjustment	Adjusts exposure time for F1 image (in milliseconds).
11	Preview F2 Image	Previews the fluorescent image: F2
12	F2 Exposure Adjustment	Adjusts exposure time for F2 image (in milliseconds).
13	Count/Speed Count Button	Initiates counting procedure.
14	Recount Button	Recounts currently loaded image.

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

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 Decluster	The command to initiate an image capture and analysis of a sample in Count/ Speed Count mode. 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 fluorescence imaging capabilities and modes of the instrument. Used to detect fluorescence properties of cells. <i>Cellometer X2 and K2 have two fluorescence channels.</i>
Brightfield Image	Image displayed on screen after it has been acquired from the brightfield optics.
Fluorescence Image F1, F2	Image displayed on screen after it has been acquired from the fluorescence optics. F1 is the first fluorescence image. F2 is the second fluorescence image.
Threshold	The limit to include or exclude a cell in a particular category (e.g., counted, not counted, live/dead, fluorescence positive/negative, etc.) based on a particular property of the cell (i.e., size, fluorescence level, bright center, etc.).



Modify 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 X2|K2 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 X2|K2 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: Cellometer instruments 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 <https://www.revivity.com/contact-us> or send email to: CellC-support@revivity.com

Servicing Hazard

No one other than Revvity-authorized personnel may service inside the protective cover of the Cellometer X2 or K2 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 Cellometer instruments 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 Cellometer instruments to the main power connection *prior* to use. Cellometer instruments 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

Cellometer instruments offer 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.

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Chapter 3. Getting Started

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



CELLOMETER X2 K2 SYSTEM COMPONENTS

The Cellometer X2 or K2 comes with the following components:

- Cellometer Instrument
- Power Supply and Power Cord
- USB 2.0 Connector Cable
- USB Drive containing Cellometer Software and PDF User Manual and Quick Start Guide
- One box of SD100 Disposable Counting Chamber Slides (75 slides for 150 counts)
- Consumable Starter Pack (includes two SD100 slides and one CCBM-015-0.5mL bead solution vial)
- One CS2-0106 AO/PI staining solution vial (sample)
- 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/BACK VIEW



Disposable Chamber Slot



Focus Adjustment



Serial# Label

Power switch

USB Input

Power adapter input

SETTING UP CELLOMETER X2 K2

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 X2 K2 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 Cellometer software must be installed BEFORE USB 2.0 cable is connected.

Note: Revvity-provided computers are shipped with Cellometer software pre-installed. No additional setup or configuration is required. If software re-installation is required, contact Support.



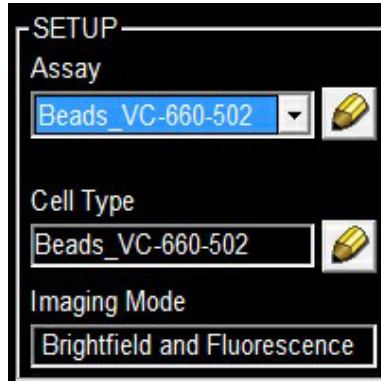
Using X2 K2 for the First Time

Before using Cellometer 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.

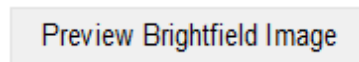
STEP 1. PREPARE SAMPLE SLIDE

- Vortex Bead Solution at low speed for 10 seconds.
- Obtain a clean Disposable Counting Chamber Slide and place on a clean Kimwipe.
- Pipette a 20 μ L 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

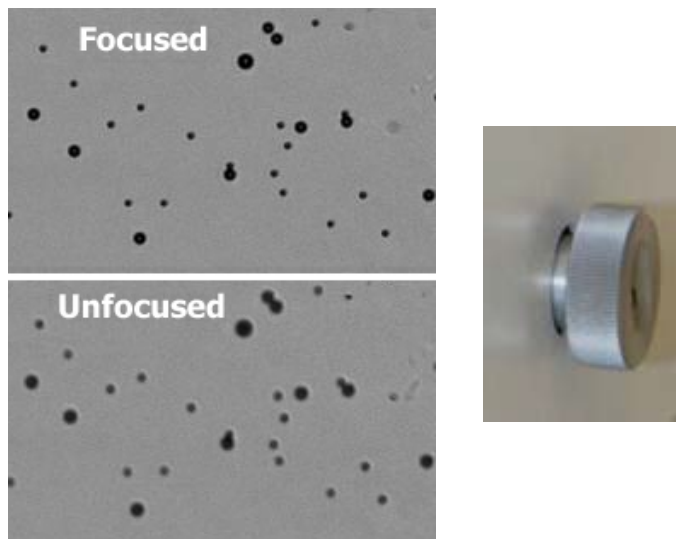


STEP 3. CLICK 'PREVIEW BRIGHTFIELD IMAGE'

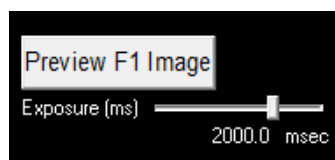


STEP 4. SLOWLY TURN THE FOCUS WHEEL UNTIL OPTIMAL CELL COUNTING FOCUS IS ACHIEVED

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



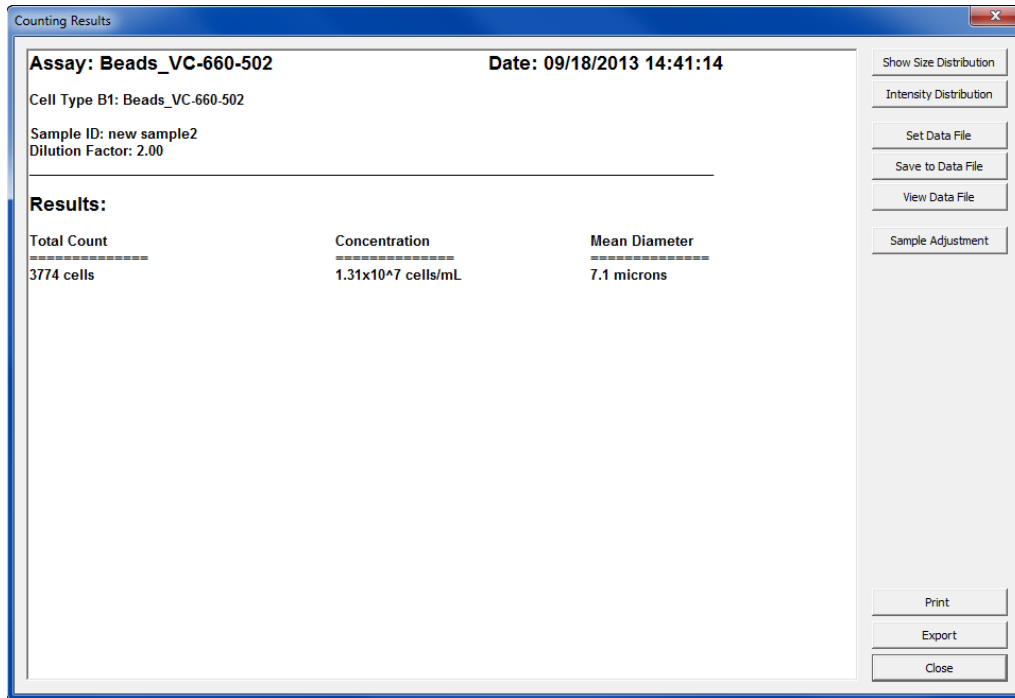
STEP 5. CLICK 'PREVIEW F1 IMAGE'



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



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



Initial setup and configuration are now complete. You are ready to start using Cellometer. 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 performing various cell-based assays using the Cellometer. 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 (x86)\Nexcelom\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.

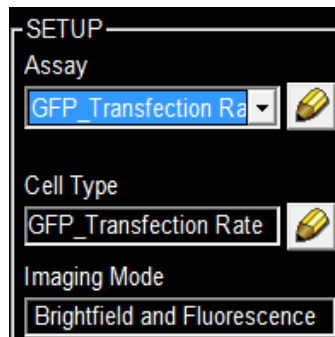
* Concentrations should be optimized for specific applications.

SAMPLE TUTORIALS

Modify Default Assay Parameters

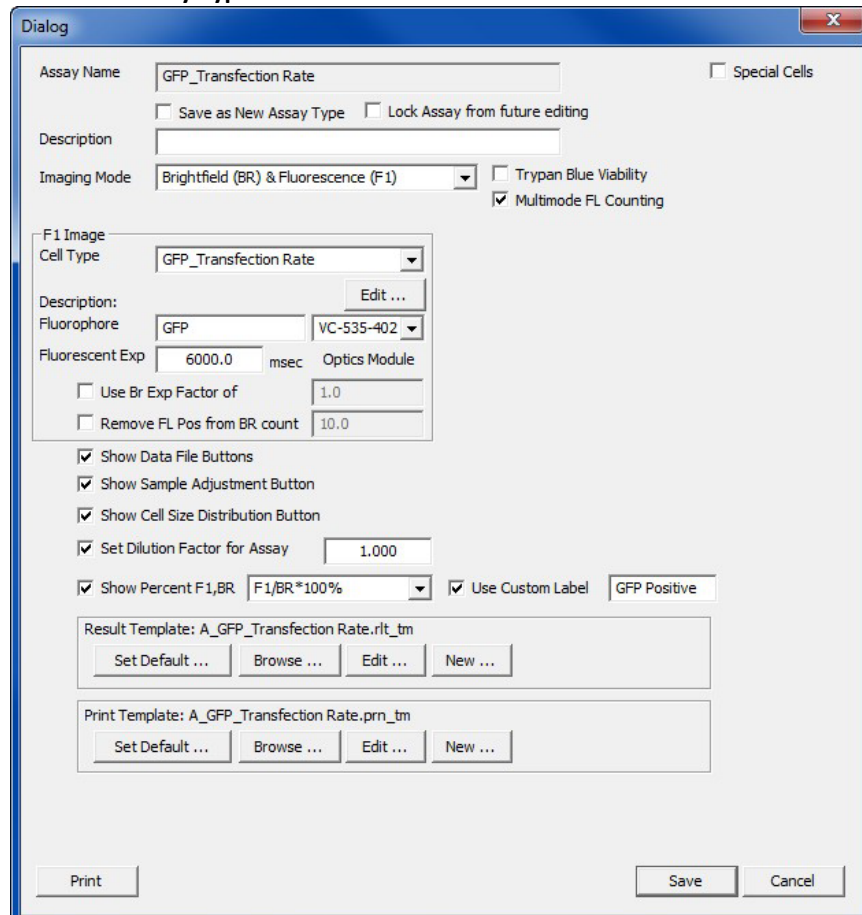
Cellometer default assay types are intended as guidelines for setting up new assays. The following is a procedure to modify assay parameter settings to suit your experiment details.

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. Check **Save as New Assay Type**.

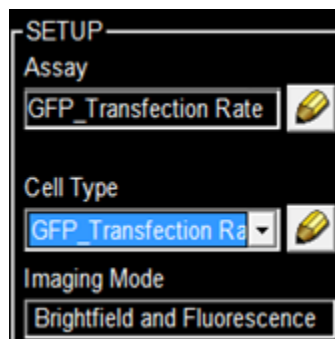


- B. Input new or modified name in **Assay Name**. *Note that **Save** button will become active once the Cell Type name has changed.*
- C. Update assay parameter settings.
- D. View and/or modify cell type parameter settings:
 - Method 1: Use **Cell Type** drop-down for a channel (e.g. *F1 Image*) to select another cell type.
 - Method 2. Click **Edit...** to edit cell type parameters for the current cell type. See *Modify Default Cell Type Parameters* on page 23.
- E. Click **Save** to save modified assay parameters.

Modify Default Cell Type Parameters

Cellometer default cell types are intended as guidelines. The following is a procedure to modify cell type parameter settings to suit your experiment details.

STEP 1. SELECT A CELL TYPE FROM CELL TYPE SETUP DROP-DOWN MENU



*You can also modify cell type parameter settings while viewing assay parameters by using the **Cell Type** drop-down for a channel (e.g., **F1 Image**) and clicking **Edit...** to view current cell type parameters.*

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

Cell Type

Cell Type Name Save as New Cell Type
 Lock from future editing

Detailed Description

Brightfield (BR) | Fluorescence (FL)

Cell Diameter Minimum 4.0 micron Maximum 50.0 micron

Roundness default: 0.10; range: 0 - 1.0; 1.0 for perfect circle

Contrast Enhancement default: 0.40; range: 0 - 0.8; high value for light cells

Decuster Parameters

Do not decluster clumps

Decuster Edge Factor default: 0.5; range 0 - 1.0; higher value for more edge enhancement

Decuster Th Factor default: 1.0; range 0 - 1.0; higher value for more sensitivity

Background Adjustment default: 1.0; range 0 - 1.0; lower value to pick up dim cells

Trypan Blue Viability Parameters

Dead Cell Diameter Minimum 4.0 micron Maximum 50.0 micron

Sensitivity default: 1.0; range 0 - 6.0; higher value to pick up more dead cells

Uniformity default: 150; range 100 - 255; higher value for non-uniform dead cells

Very Dim Dead Cells

Print Save Cancel

- A. Check **Save as New Cell Type**.
- B. Input new or modified **Cell Type Name**. *Note that **Save** button will become active once the Cell Type name has changed.*
- C. Update cell type parameter settings.
- D. Change Fluorescence exposure time if needed.
- E. Click **Save** to save modified cell type parameters.

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.

File > 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

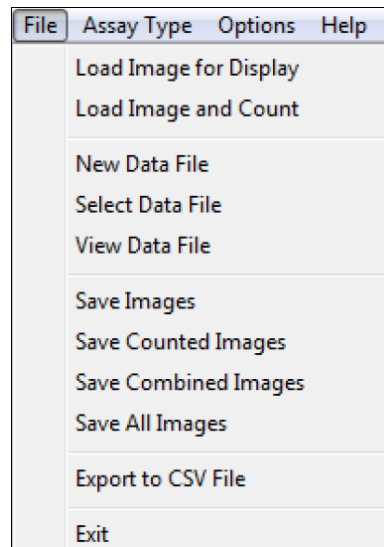
Save cell images with outlines indicating counted objects.

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 > Exit

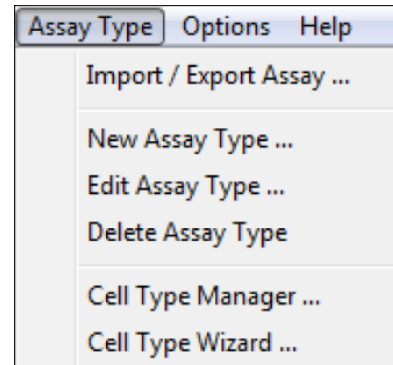
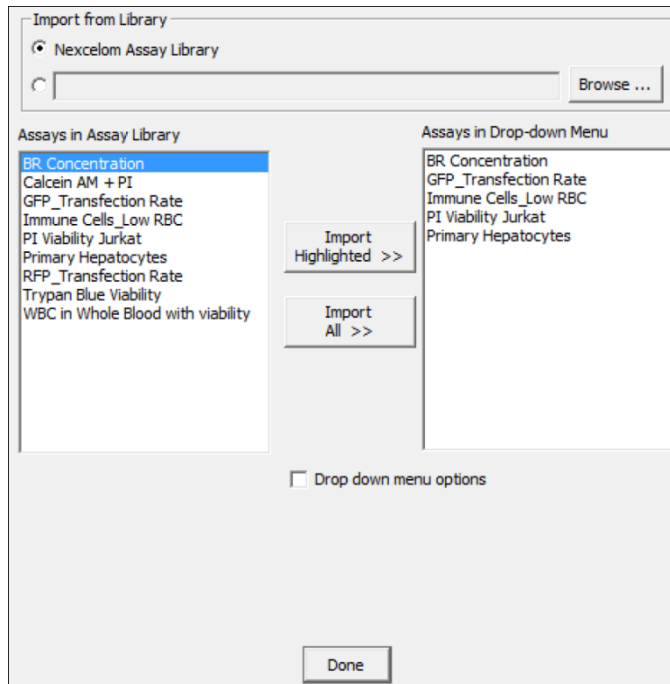
Quit Cellometer software.



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 for user to load a saved Assay Library.

Assays in Assay Library: List of Assay Types in an Assay Library.

Assays in Drop-down Menu: List of Assays in the Cellometer software Setup Assay Drop-down list.

Import Highlighted: Import highlighted assay type in a selected assay library (on the left) to the Assay Drop-Down Menu (on the right).

Import All: Import the complete list of Assays from the Assay Library to the drop down menu.

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 the assay types in the drop down menu to a new Assay Library.

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

Done: Close Assay Manager window.

Assay Type -> Edit Assay Type

Assay Name: GFP_Transfection Rate Special Cells

Save as New Assay Type Lock Assay from future editing

Description: [Empty]

Imaging Mode: Brightfield (BR) & Fluorescence (F1) Trypan Blue Viability Multimode FL Counting

F1 Image

Cell Type: GFP_Transfection Rate

Description: [Empty] Edit ...

Fluorophore: GFP VC-535-402

Fluorescent Exp: 6000.0 msec Optics Module

Use Br Exp Factor of 1.0

Remove FL Pos from BR count 10.0

Show Data File Buttons

Show Sample Adjustment Button

Show Cell Size Distribution Button

Set Dilution Factor for Assay 1.000

Show Percent F1, BR F1/BR*100% Use Custom Label GFP Positive

Result Template: A_GFP_Transfection Rate.rit_tm

Print Template: A_GFP_Transfection Rate.prn_tm

Print Save Cancel

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 the new Assay Type (See *Modify Default Assay Parameters* on page 22 for details).

Description: Text descriptions for your assay.

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

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

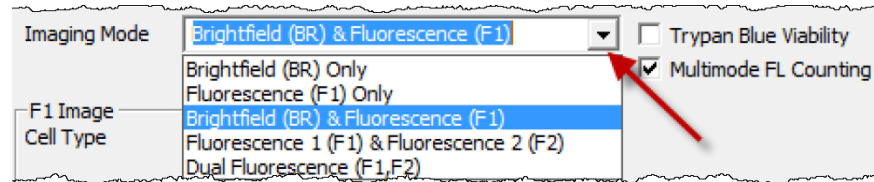
Special Cells

Slide Type: CHT4-xD100

Adipocyte

CM

Low Conc.

Imaging Mode:

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

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

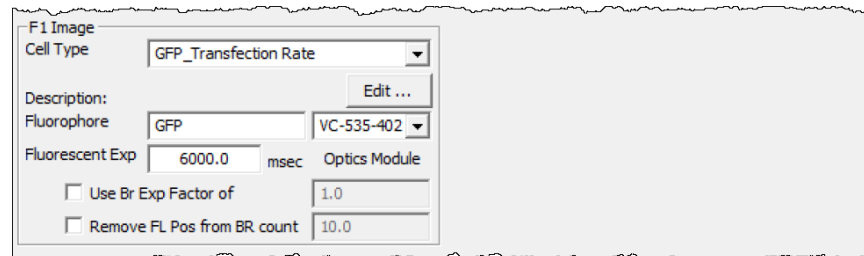
Brightfield (BR) and Fluorescence (F1): Acquire both brightfield and fluorescence images. Cell counting results are produced using a combination of both images.

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

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

Trypan Blue Viability: Check for trypan blue viability (available only in *BR Only* or *BR&F1* modes).

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



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

F2 Image Area: *Not shown; visible in 'Fluorescence 1 and Fluorescence 2' imaging mode.* Contains Cell Type and Fluorescence parameters for the F2 image.

Cell Type: Drop-down menu to select cell type parameters (See *Modify Default Cell Type Parameters* on page 23 for details).

Edit: Edit selected cell type parameters.

Fluorophore: User-defined name of fluorophore used in this assay (Note: VC-535-402 or VC-660-502 indicates the fluorescence optics module that detects that particular fluorophore).

Fluorescent Exp: Fluorescence exposure time in milliseconds.

Show Report Format: Select to display Counting Results display option buttons that appear on the right hand side of the Counting Results screen.

The following options allow you to customize how results are displayed in the Counting Results screen:

Show Cell Count: Check to display cell counts.

Show Trypan Blue Dead Cell Count: Check to display dead cell counts; *enabled based on assay type.*

Show Trypan Blue Viability: Check to display the % viability; *enabled based on assay type.*

Show Cell Mean Diameter: Check to display the mean diameter of counted cells.

Show Trypan Blue Dead Cell Mean Diameter: Check to display the mean diameter of trypan blue stained dead cells; *enabled based on assay type.*

Show Dilution: Check to display the dilution factor entered in the main window.

Show Concentration: Check to display the cell concentration.

For Fluorescent Positive Cells, Use Brightfield Cell Size for Histogram: Check to use the brightfield cell size when generating the cell size histogram for fluorescent cells.

Separate Fluorescent Positive/Negative Cells for Cell Size Histogram: Check to generate a cell size histogram with FL positive cells shown as green bars and FL negative cells as red bars.

Show Percent F1/BR: Indicate how result is calculated. Ex: To show percentage of FL positive cells, choose $F1/BR*100\%$. To show percentage of FL negative cells, choose $(BR-F1)/BR*100\%$. Note that additional calculations appear for *F1&F2* or *Dual fluorescence* modes.

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

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

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

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

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

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

Save Trypan Blue Dead Cells to Data file: Check to save dead cell counts; *enabled based on assay type.*

Set Dilution Factor for Assay: Enter final dilution factor for assay.

Print: Print out selected Assay Type parameters.

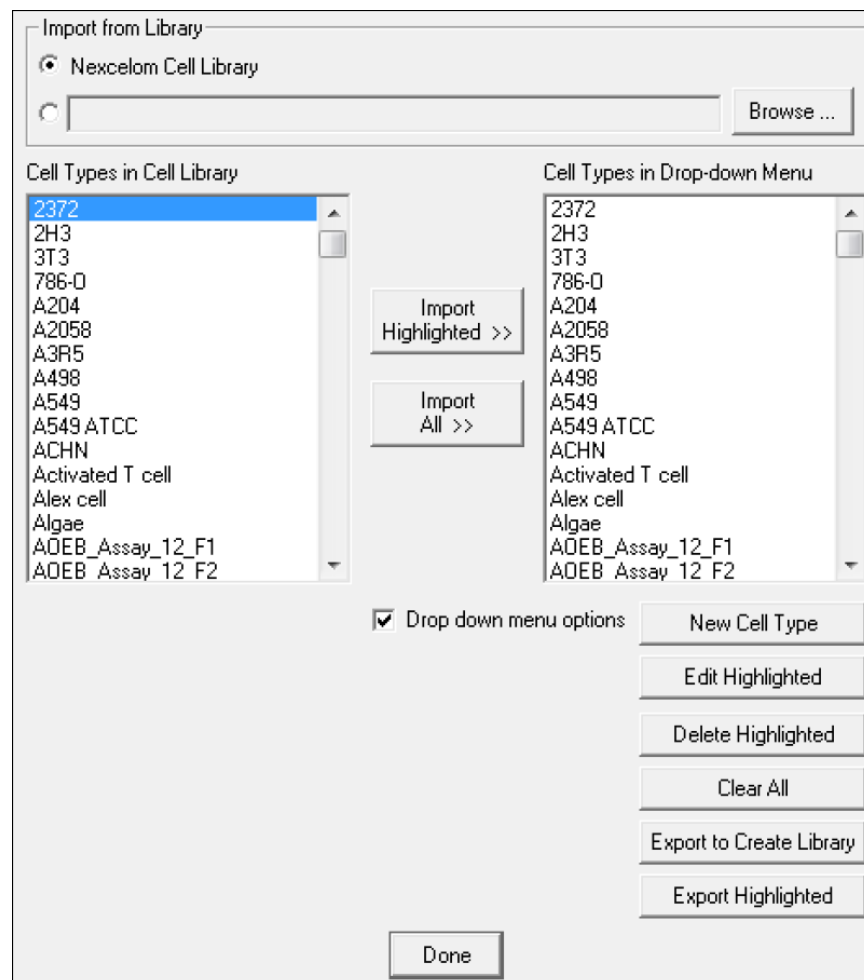
Cancel: Close Assay Type without saving changes.

Save: Saves 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 on the left.

Import All: Import all cell types from the Cell Library on the left.

Drop down menu options: Check to see details.

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

Edit Highlighted: Edit the highlighted cell type.

Delete Highlighted: Delete the highlighted cell type.

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

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

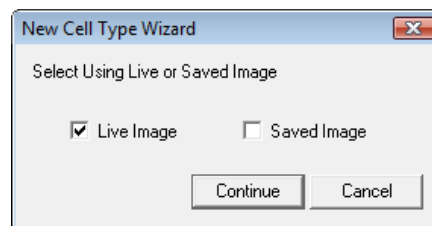
Export Highlighted: Export only the highlighted cell type in drop-down menu to new Cell Type Library.

Done: Close Cell Type Manager window.

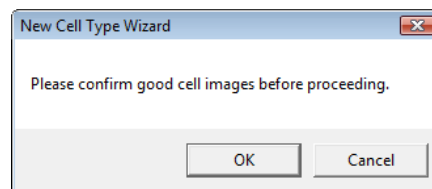
Assay Type > Cell Type Wizard

The Cell Type Wizard is a tool that guides the user in creating a new Cell Type Parameter for a cell sample. Using a cell sample loaded in the CHT4 counting chamber or a saved image of the cell sample the Cell Type Wizard is able to analyze and create parameters for accurately counting the cells. The user has the ability to name the resulting parameters and save them for future counts.

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



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



Cell Type Wizard processes image 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 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 with 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 in order 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 the 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 the minimum diameter of a trypan blue stained cell that will be included in the count.

Dead Cell Diameter Maximum: Value indicates the 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 there are cells with light and dark areas of staining.

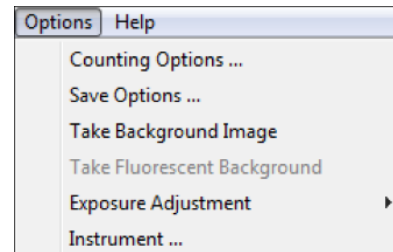
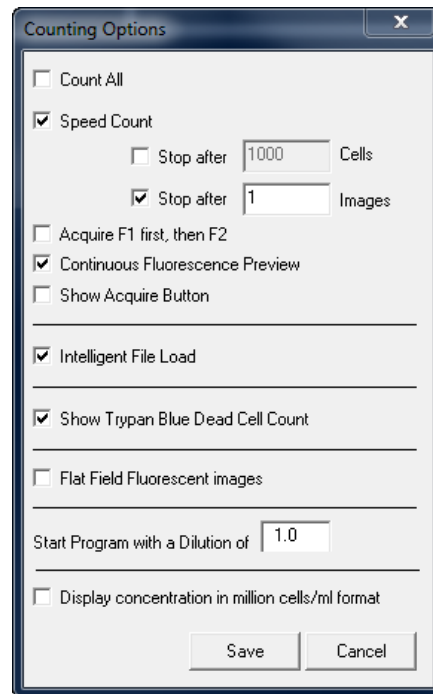
Suggested value: 150; Range 100 to 255 100 (Cell uniformly stained with trypan blue)

255 (Cell has dark and light areas stained with trypan blue)

Very Dim Dead Cell Enhancement: Checking this box indicates that the software uses a special function 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: Stop 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: Allow user to adjust focus in Preview F1 Image mode.

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

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

Flat Field Fluorescent images: Allow fluorescence background image to be used for data analysis (FL reference slides needed).

Start Program with a Dilution of: Allow 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: Close Counting Option and save changes.

Cancel: Close Counting Option menu 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 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 will auto export 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.

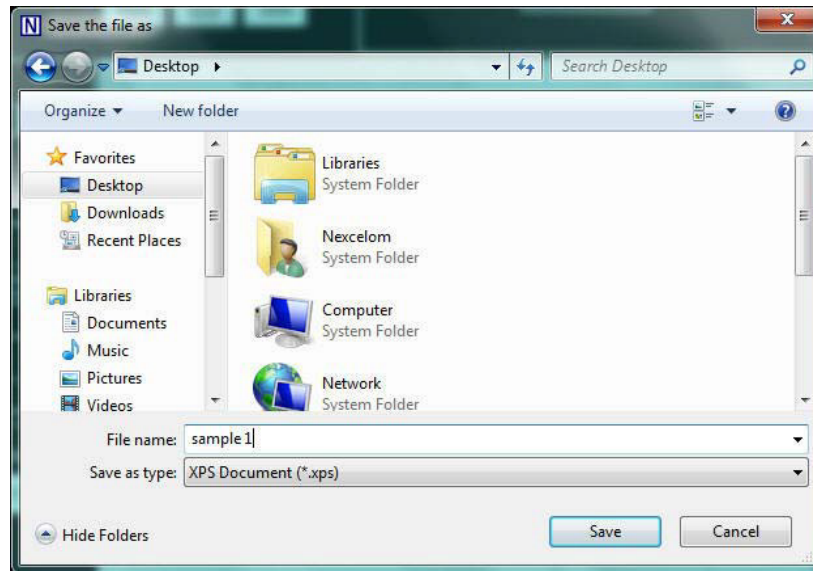
Auto Print Count results: Click **Save** button.

- **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.

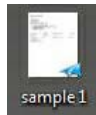


If not connected to a printer, the print dialog will automatically select the default system printer. *For Windows 7 Operating system *.xml is the default printer.*


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.revvy.com/contact-us> or send email to: CellC-support@revvy.com

 03/11/2011 14:32:49		
Sample ID: HCT116_GFP		
Dilution: 1.00		
Cell Type: Cell Line_Assay_03		
Instrument Serial Number: Vision-202-0012		
Chamber Type: CHT4-xD100		
Operator Signature:		
<hr/>		
Results: Cell Concentration via Brightfield and Fluorescence		
Count	Concentration	Mean Diameter
=====	=====	=====
Brightfield: 992	8.97x10 ⁵ cells/mL	12.2 micron
Fluorescence 1: 596	5.38x10 ⁵ cells/mL	12.0 micron
<hr/>		
Results: Calculations		
% GFP Positive		
Equation Results: 60.0%		
Fluorescence 1 Mean Intensity: 5039.7 Counts		

Options > Take Background Image

Take brightfield background image of Cellometer system without cell counting chamber. For Cellometer X2 and Cellometer K2 instruments, background images are needed for both Fluorescence Optics Modules. Brightfield exposure times will automatically be set and saved.

Options > Take Fluorescence Background

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

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 Cellometer software is closed and restarted, the saved exposure time will be used.

Options -> Instrument

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

Instrument: Indicates name of 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.

1 FOM Installed/2 FOM Installed: Indicates instrument for which software is configured (Factory Setting).

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

HELP MENU

Help> Cellometer Help

Displays user manual and available tutorials.

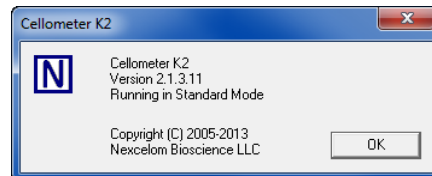
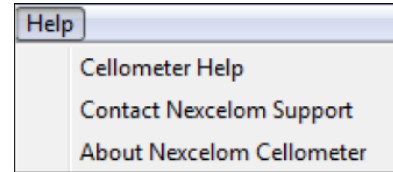
Help>Contact Nexcelom Support

Opens up Cellometer Support web page.

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

Help> About Nexcelom Cellometer

Displays version number of Cellometer software currently installed.



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Chapter 6. Technical Information

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

INSTRUMENT SPECIFICATIONS

Instrument Specifications

- Weight: 23.0 lbs (10.4kg)
- Dimensions: 6.0'' x 8.5'' x 14.0'' (15.2 cm x 21.6 cm x 35.6 cm)
- Input to Power Adaptor: 100-240 AC, 50-60 Hz, 1.0 A
- Output to Instrument: 12 VDC, 3.34 A

Declaration of Conformity

Cellometer X2 K2 conforms to appropriate country standards and governing regulations as listed in the *Declaration of Conformity*. To request the X2 K2 *Declaration of Conformity*, contact Support by visiting <https://www.revivity.com/contact-us> or send email to: CellC-support@revivity.com

ENVIRONMENTAL REQUIREMENTS

Environmental requirements for the intended operation of Cellometer 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 $\pm 10\%$ 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.revivity.com/contact-us> or send email to: CellC-support@revivity.com

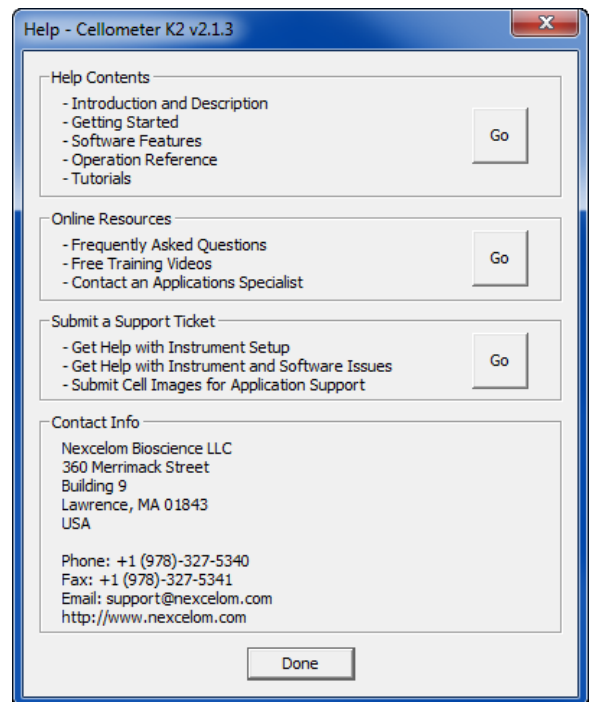
ONLINE SUPPORT RESOURCES

Revvity offers online Support resources directly from the Cellometer 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.revivity.com/contact-us> or send email to: CellC-support@revivity.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 leading up to the error, if possible.
3. If necessary, 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 X2 and K2 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 μ L 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. *Reagents and Kits available for Cellometer K2 vary from those available for Cellometer X2 instruments.*

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

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