USER MANUAL

CELLOMETER™ ASCEND

FLUORESCENT CELL COUNTER

8004708 Rev A

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

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Cellometer™ Ascend User Manual

8004708 Rev A

May 2024

Table of Contents

CHAPTER 1. INTRODUCTION 1

Cellometer Ascend Instrument Overview 1

Intended Use 1

Contents of Shipping Container 2

Matrix Software Overview 2

Matrix 21 CFR Part 11 Module Overview 3

About this User Manual 4

Glossary of Abbreviations 5

Glossary of Symbols 6

CHAPTER 2. HAZARDS, SAFETY, AND ENVIRONMENT 7

Potential Hazards 7

Electrical Hazard 7

Instrument System Hazard 8

Servicing Hazard 8

Safety Information 9

Safety Protocols 9

Safety Features 9

Environmental Requirements 10

CHAPTER 3. INSTRUMENT DESCRIPTION 11

Ascend Components 11

Device SN Label 11

Instrument Specifications 12

Technical Information 13

Declaration of Conformity 13

CHAPTER 4. UNPACKING AND SITE PREPARATION 15

Unpacking and Setting Up Instrument 15

Site Preparation 16

Facility Requirements 16

Transporting the Instrument 16

Disposal of Waste Electrical and Electronic Equipment 16

Instrument and Software Validation 17

Cellometer Ascend IQ/OQ Validation 17

21 CFR Part 11 OQ Validation 17

CHAPTER 5. OPERATION 19
Launching the Software 19
Matrix Screen Elements 19
Navigation Bar 20
Workflow Tabs 20
Simplified Workflow 22
User Favorites 22
Streamlined User Experience 22
Focus Methods 23
Image-Based Auto Focus 23
Slide-based Auto Focus 23
Viewing Software Version 24

CHAPTER 6. VIABILITY METHODS 25
Evaluating Viability Methods 25
Using Trypan Blue Viability Method 25
Preparing a Cell Sample for Trypan Blue Viability Determination 25
Trypan Blue Staining Solution Guidelines 25
Using AO/PI Viability Method 26
Preparing a Cell Sample for AO/PI Viability Determination 26
AO/PI Staining Solution Guidelines 27

CHAPTER 7. SAMPLE PREPARATION 29
Preparing Counting Chamber Slides 29
Loading Samples into Counting Chambers 29

CHAPTER 8. MATRIX COUNTING AND ANALYSIS WORKFLOW 31
Performing a Count 31
Choosing Data Acquisition Workflow 31
Selecting a Favorite 31
Entering Parameter Settings 32
Loading Samples 33
Preview Mode 34
Previewing Live Images 34
Adjusting Focus 34
Previewing Channel Images 35
Count Mode 36
Analyzing Scan Results 37
Understanding Default Report Tabs 37
Changing Well View Image Display 38

Varying Well View Channels/Counted Overlay Display 38
Understanding Custom Reporting 38
Exporting/Printing Scan Result Data 39
Verifying Auto Export 39
Exporting and Printing Scan Results (Manual Settings) 40
Performing a Recount 41
Refining Assay Details/Selecting New Assay 41
Managing Channel Mappings 41
Modifying Auto Export Options 42
Tapping the Recount Button 42
Best Practices and Workflow Tips 43

CHAPTER 9. CLEANING, MAINTENANCE AND STORAGE 45

Cleaning 45
Routine Maintenance 45
Storage 46

CHAPTER 10. TROUBLESHOOTING AND FAQS 47

Troubleshooting and Instrument Messages 47
Frequently Asked Questions 48

CHAPTER 11. REVVITY SUPPORT 49

Scope of Support Services 49
Contact Methods 49
Reporting an Issue to Support 49
Generating Diagnostic Reports 50

APPENDIX A. CONSUMABLES 51

Counting Chamber Slides 51
Assay Reagents and Kits 51

APPENDIX B. WARRANTY AND LICENSE DETAILS 53

Warranty Information 53
Terms and Conditions 53
Limitation of Liability (Hardware and Software) 54

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Chapter 1. Introduction

This chapter presents introductory information to be reviewed *prior* to unpacking your instrument. It includes Cellometer™ Ascend product and Matrix™ software overviews, lists contents of shipping container, and identifies symbols/abbreviations used on instrument device label and in this user manual.

CELLOMETER ASCEND INSTRUMENT OVERVIEW

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

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

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

An optional Matrix *21 CFR Part 11* module ensures the integrity of your data by limiting access to authorized users, retaining an audit trail of user transactions, offering electronic signing/unsigning capabilities and providing multi-layer access control via user roles.

Intended Use

Intended use of the Cellometer Ascend is to count cell lines and primary cells (e.g., total nucleated cells, PBMCs, splenocytes, hepatocytes, stem cells, tumor/tissue digests, etc.) with the ability to determine cell count, viability and additional analysis using trypan blue exclusion or fluorescent reagents.

Personnel operating this instrument are encouraged to familiarize themselves with device controls and operation. Ensure that users can identify all components associated with the instrument, perform adequate adjustments and understand performance criteria. If issues are encountered, see *Chapter 10. Troubleshooting and FAQs* on page *47* to restore performance if instrument does not meet or exceed defined performance criteria.

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

CONTENTS OF SHIPPING CONTAINER

Cellometer Ascend Instrument

Power Supply and **Power Cord**

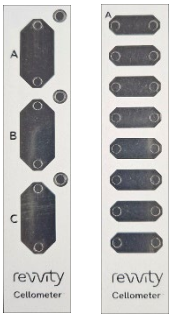
Matrix Software (pre-installed on instrument)

USB Drive containing PDF files listed below:

- *8004707 Cellometer Matrix Software User Manual*
- *8004708 Cellometer Ascend User Manual (this document)*
- *8004709 Cellometer Ascend Quick Start Guide*
- *8004710 Cellometer Ascend Focus Guide*

USB Drive

Ascend Slides



Box of 100 **3-Chamber Counting Chamber Slides** (ASD-CHM3-001)

Five **8-Chamber Counting Slides** (not for individual sale; ASD-CHM8-001 for a box of 100)

One 0.5 mL vial of **Fluorescent Counting Beads** (not for individual sale; CCBM-015-0.5mL)

One 1.0 mL vial of **AO/PI Viability Reagent** (not for individual sale; CS2-0106-5mL for a 5 mL vial)

MATRIX SOFTWARE OVERVIEW

The Matrix software used to run the Cellometer Ascend is shared by multiple product families and includes default assays (identified with *ASD_*, *K2_*, *MX_*, or *PLX_*instrument prefixes) and report templates for *all* supported product types. However, only assays available for the Ascend will be displayed when entering setup details, and report templates will be adjusted dynamically for presentation based on scan results. Assays and report templates for *all* product types can be viewed in the **Manage** tab > *Assays* and *Report Templates* screens.

Key elements in Matrix software functionality are described below:

- *Acquiring Sample Data* – The **Acquire** tab > *Setup* and *Preview* options are sequential screens used to acquire sample data. After loading samples, entering setup details, selecting at least one well and then tapping the **Preview** button, you can preview live channel images and adjust instrument focus/fluorescent exposure for each channel. Tapping the **Count** button engages the camera to acquire sample images as specified by the selected assay, which are then processed by the Matrix imaging and pattern-recognition software to decluster, identify and count individual cells according to defined cell type parameters.
- *Analyzing Scan Results* – The **Data** tab > *Select*, *Results* and *Recount* options are sequential screens used to analyze scan results. Once a result is selected, you can vary the image displayed by tapping available channel buttons (e.g., **BR**, **FL1**, **FL2**, etc.) appearing across the top of the **Well View** tab or by choosing another well. Data associated with the well appears below the image or can be viewed as a consolidated report including data from *all* wells by tapping the **Summary** tab. To fine-tune the analysis of a scan, tap the **Recount** button and either select a new assay or tap the **View** button for the current assay to edit parameters as necessary. *Last Used Assay parameters are provided for your reference*.

- *Setting Up Auto Exports* – The Matrix auto export feature allows you to select the *Images* (e.g., *Raw Images* and *Colorized Images*), *Data* (e.g., *Well Level CSV*, *Object Level CSV* and *Object Level ACS*) and *Archive* (e.g., *Data Set*) output file types to be exported for an assay after completion of scan analysis. In addition, ACS templates can be imported into the Matrix software and assigned to an assay. On export, data will auto populate into the specified ACS template and can launch FCS Express if installed on your system and if the *Auto Open* option is also selected.
- *Customizing Reports* – The Matrix custom reporting feature allows you to assign and/or modify report templates to be used by assays when generating scan results. You can change the report template used for displaying **Well View** tab data and manage the *Reports List* associated with an assay. You can also enable up to five additional tabs or specify output file types (e.g., *CSV*, *Excel*, *PDF* and *Word*) to be automatically exported, opened or printed after completion of scan analysis.
- *Managing Assays, Cell Types, ACS Templates and Report Templates* – The **Manage** tab > *Assays*, *Cell Types*, *ACS Templates* and *Report Templates* options display lists of these entities currently loaded in your instrument system. From these screens you can import/export, rename, delete or show/hide an entity in its applicable list. For most entities you can also view, create or use locked entities as templates to create new ones.

Matrix Screen Elements on page *19* and *Chapter 8. Matrix Counting and Analysis Workflow* on page *31* are the only sections in this manual that reference using the Matrix software. See the *Cellometer Matrix Software User Manual* for a full description of Matrix software functionality.

MATRIX 21 CFR PART 11 MODULE OVERVIEW

The Matrix software offers a *21 CFR Part 11* module that is compliant with the *Code of Federal Regulations (CFR) Title 21 Part 11 – Electronic Records: Electronic Signatures* published by the U. S. Food and Drug Administration (FDA). In summary, this module ensures that an organization's use of electronic records and digital signatures to replace traditional paper-based documentation complies with current FDA regulations.

Key elements in Matrix *21 CFR Part 11* module functionality are described below:

- *Electronic Signatures* are captured during the counting and analysis workflow using **e-Sign**/**e-Unsign** buttons and include the name of an authorized user, date/time when the signature was executed, reason for the action being performed and meaning of the signature (i.e., for the *Review*, *Approval* or *Rejection* of an action). If a user's assigned role does not have permissions to approve count results, a supervisor can log in to e-Sign the record on behalf of the user.
- *Electronic Records* are created by linking electronic signatures to user actions ensuring that records have not been copied or falsified in any manner.
- An *Audit Trail* provides assurance regarding the integrity of an electronic record and continually monitors all users performing actions, the type of actions performed, and the date/time associated with user actions.

This module may be enabled for your instrument at time of purchase or implemented as an upgrade in the field by Support. See the *Cellometer Matrix Software User Manual* for a full description of Matrix *21 CFR Part 11* module functionality.

ABOUT THIS USER MANUAL

This *Cellometer Ascend Matrix User Manual* provides information on the following topics:

- Instrument and Software Overviews
- Potential Hazards and Safety Information
- Components, Specs and Environmental
- Unpacking and Site Preparation
- Installation, Operation and Screen Elements
- Comparison of Viability Methods
- Sample Preparation
- Matrix Counting and Analysis Workflow
- Cleaning, Maintenance and Storage
- Troubleshooting, Messages and FAQs
- Contacting Support and Reporting Issues
- Consumable Slides, Reagents and Beads
- Warranty and License Details

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

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

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

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

GLOSSARY OF ABBREVIATIONS

The following abbreviations may be displayed on the shipping container, on the Cellometer Ascend 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 be displayed on the shipping container, on the Cellometer Ascend 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. Located in User Manual**		**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**
	**Australian RCM Mark**				

Chapter 2. Hazards, Safety, and Environment

As with any equipment that involves moving parts, there are potential hazards involved in this instrument's operation and maintenance. This chapter identifies potential hazards, lists safety information and provides environmental requirements for intended operation.

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

Electrical Hazard

No part of the exterior housing should be removed. Do *not* open the instrument cover. For assistance, contact Support by visiting https://www.revvity.com/contact-us or send an 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 it. Damaged instruments or cables may result in injury to the user.

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

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

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

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

Servicing Hazard

No one other than Revvity-authorized personnel may service inside the protective cover of the Cellometer Ascend 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 Ascend 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 due to 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 Ascend instrument to the main power connection *prior* to use. The Ascend 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 Ascend instrument offers several safety features to prevent its misuse or unintentional activation. All personnel who operate the system should be familiar with these safety features.

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

Federal Communications Commission

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

ENVIRONMENTAL REQUIREMENTS

Environmental requirements for intended operation of the Cellometer Ascend instrument are presented below.

- For Indoor Use Only
- Elevation: 0 to 2,000 m
- Temperature Range: 10 °C to 30 °C
- Relative Humidity: 0% to 90% RH, non-condensing
- Pollution degree: Degree 2
- MAINS supply voltage fluctuations up to ± 10% of the nominal voltage

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

Chapter 3. Instrument Description

This chapter describes the Cellometer Ascend instrument components, device label and system specifications.

ASCEND COMPONENTS

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

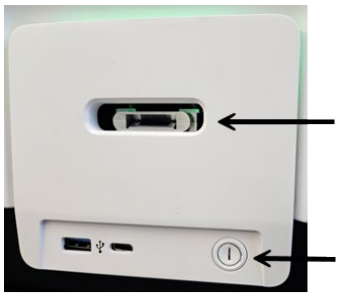
Ports: One port on front of instrument (USB A). Three ports on back of instrument (two USB A and one Ethernet).

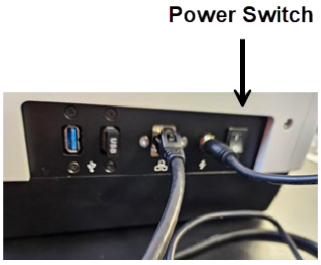
Power Button: Toggles instrument from *Run* mode (power *ON*) to *Standby* mode (power *OFF*).

Power Switch: Controls power going to instrument.

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

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





DEVICE SN LABEL

Polarity DC Power Connector

Serial Number

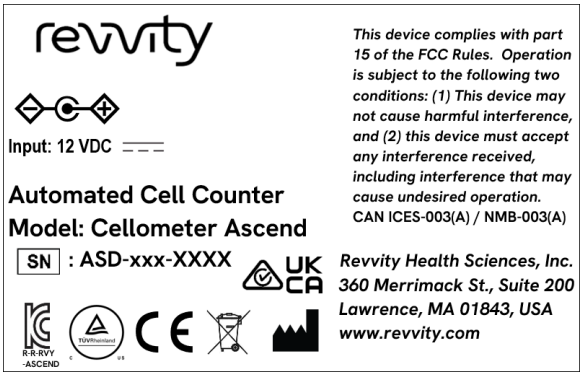
Australian RCM Mark

UK Conformity Assessment Declaration of Conformity to UK Directives

Korean Certification for EMC Directives and Registration Number

NRTL Safety Mark

European Conformity Mark



Waste Electrical and Electronic Equipment Directive (WEEE)

Manufacturer

INSTRUMENT SPECIFICATIONS

The *Cellometer Ascend* is your all-in-one automated cell viability counter. Using both *Brightfield* and *Dual-Fluorescent* imaging in combination with pattern-recognition software, it quickly and accurately identifies and counts cells. For cultured cells stained with trypan blue or fluorescent reagents, the Ascend simultaneously calculates *Live/Dead* and *Total* cell counts, *Viability (%)*, and *Live* and *Total* cell concentrations, including a helpful graphic overlay used to highlight live and dead cells with color-coded outlines in the image being viewed.

	Cellometer Ascend	
Channels	Brightfield and Fluorescent (FL1 - *Green* and FL2 - *Red*)	
Fluorescent Optics	Excitation / Emission: 470 nm / 534 nm *Example Fluorophores:* - Acridine Orange (AO) - Calcein AM - CFDA - GFP (Green Fluorescent Protein) - SYTO™ 9 and SYTO™ 13	Excitation / Emission: 531 nm / 655 nm *Example Fluorophores:* - Propidium Iodide (PI) - Ethidium Bromide (EtBr)
Commonly Used Compatible Dyes	AO/PI, Trypan Blue	
Counting Speed (Per Sample)	<30 sec	
Volume (Per Chamber)	3-Chamber Slide: 20 µL 8-Chamber Slide: 10 µL	
Cell Size/Diameter Range	4 – 90 µm	
Concentration Range	$1x10^5$ – $1x10^7$ cells/mL	
Weight	24 lbs (10.5 kg)	
Width	9.9” (25 cm)	
Depth	13” (32.8 cm)	
Height	18.7” (47.5 cm)	
Input to Power Adapter	100-240 VAC, 50/60 Hz, 1.5 A	
Output to Instrument	12 VDC, 10 A	

The Cellometer Ascend uses brightfield and fluorescent imaging with pattern-recognition software to quickly and accurately decluster, identify and count individual cells.

TECHNICAL INFORMATION

The following table presents Cellometer Ascend technical information including integrated computer and camera details, and environmental requirements.

	Cellometer Ascend Cell Viability Counter	
Display	10.1”, 1024 x 768 TFT with LED back light	
Operating System	Windows 10 LTSC	
External Connectors	*On Instrument Front:* 1 USB A Port	*On Instrument Back:* 2 USB 2.0 Ports 1 Ethernet Port 1 Power Input Plug
External Button	*On Instrument Front:* Push Button for Power activates Light Bar above Sample Slot	*On Instrument Back:* Flip Switch for Power
Circulation and Cooling	Allow at least 6 inches of unobstructed air circulation around vents on the instrument while in use to ensure adequate cooling.	

DECLARATION OF CONFORMITY

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

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Chapter 4. Unpacking and Site Preparation

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

UNPACKING AND SETTING UP INSTRUMENT

Congratulations on your purchase of one of the best declustering cell counters available! Please ensure you have secured the space required to set up your new Cellometer Ascend (see *Facility Requirements* on page *16*).

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

1. Inspect the package to ensure no damage has occurred during shipping, if applicable. Contact Support if damage has visibly affected the instrument.
2. Ensure the box is facing up (i.e., *This End Up* symbol is facing in the right direction). If not, carefully turn the box right side up. *Box will weigh approximately 28 lbs (12.7 kg).*
3. Open the outer box and remove protective packaging.
4. Remove the Consumables box and carefully open to locate any temperature-controlled beads/reagents. *Storage temperature is printed on outer bag containing the reagent, as well as on the vial label. Scan QR code on product card provided for additional reagent information.*
5. Remove instrument accessories (e.g., Power Supply/Cord, USB Drive, *Cellometer Ascend Focus Guide*, printed versions of user manuals, as well as any other documents) and set aside.
6. Remove the Ascend instrument and place it in the prepared area (see *Facility Requirements* on page *16*).
7. Remove the protective plastic around the Ascend.
8. Connect the Power Supply to Power cord.
9. Connect the Power Supply to the instrument (via input on back panel).
10. Plug the Power Cord into a surge protector (*recommended*) or dedicated electrical outlet.
11. Turn the instrument Power Switch (*located on lower left side of back panel when facing the front of the instrument*) to *ON* position.
12. Press the Power Button (*located on front panel*) to power *ON* the instrument. *Light bar on front of instrument will be lit and internal Operating Computer will run through startup sequence.*

The system is now ready for use.

SITE PREPARATION

Facility Requirements

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

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

Keep the area around the Cellometer Ascend clean between, during and post operation.

Electrical requirements for the Cellometer Ascend are as follows:

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

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

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

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

Transporting the Instrument

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

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

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

Disposal of Waste Electrical and Electronic Equipment

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

INSTRUMENT AND SOFTWARE VALIDATION

Cellometer Ascend IQ/OQ Validation

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

21 CFR Part 11 OQ Validation

If you have enabled Matrix *21 CFR Part 11* module functionality for your system, a separate *OQ* validation has been designed as a supplement to be run in conjunction with the *Cellometer Ascend IQ/OQ Protocol*.

Note: Integrating the *Matrix 21 CFR Part 11 OQ Protocol Supplement* into the *Cellometer Ascend IQ/OQ Protocol* requires that you have purchased a Matrix *21 CFR Part 11* module license and the *CMT-ASD-21MX-IQOQ Kit*.

Our experienced Support team is available to assist your organization during set up of the instrument and while performing the IQ/OQ validation. Contact Support by visiting https://www.revvity.com/contact-us or send an email to: CellC-support@revvity.com

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Chapter 5. Operation

This chapter describes launching the Matrix software, presents basic screen elements, and provides details on how to view the installed software version.

As the Ascend is a touch-screen device, tap gently on screen elements (e.g., tabs, icons, dropdowns, or buttons) using a finger or stylus to make software graphical user interface (GUI) selections. *As an alternative, a USB mouse and keyboard may be connected.*

WARNING: Never place a foreign object inside the Sample Slot (which is designed to accept only one slide at a time). Should anything get stuck or spill inside the sample slot, power OFF the instrument and contact Support for service (see page *49*).

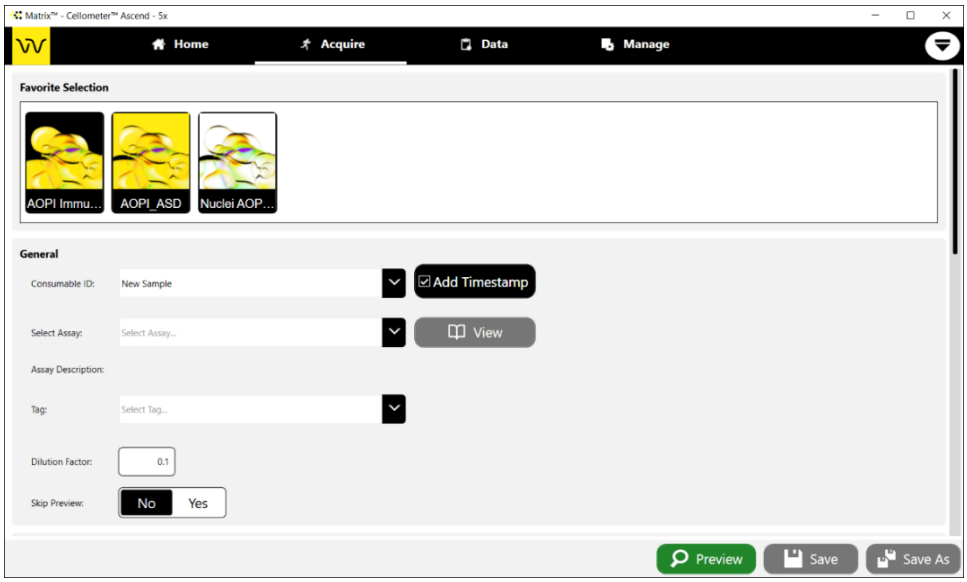
LAUNCHING THE SOFTWARE

To power on instrument, turn Power Switch (located on lower left side of back panel when facing the instrument) to *ON* position and press the Power Button (located on front panel). Light bar on front of instrument will be lit.

On the tablet touchscreen desktop, use your finger or a stylus pen to double-tap the Matrix icon to launch the software.

MATRIX SCREEN ELEMENTS

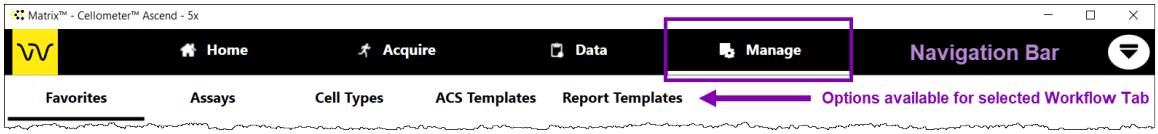
Upon launch of the software, you are presented with the first screen (*Setup*) in the data acquisition workflow. *This workflow is available at any time by tapping the **Acquire** workflow tab.* Basic screen elements are described below.



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

Navigation Bar

The *Navigation Bar* visible across the top of the screen is always displayed and contains the **Home**, **Acquire**, **Data** and **Manage** tabs. Tapping on a tab displays options for that tab (if available) in a secondary *Options Bar*.

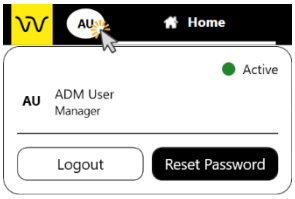


Functionality of Navigation Bar elements is described below.

Workflow Tabs: The **Home**, **Acquire**, **Data** and **Manage** tabs represent key areas of functionality as described in *Workflow Tabs*, below. Tapping a tab may display options for that tab (if available) in a sub navigation bar.

If the *Matrix 21 CFR Part 11* module is enabled, additional **Roles**, **Users**, and **Audit Trail** tabs are displayed.

User ID/User ID Card: If the Matrix *21 CFR Part 11* module is enabled, the identity of the currently logged in user AU is displayed. Tapping the User ID displays the User ID Card which contains the **Logout** and **Reset Password** buttons. See the *Cellometer Matrix Software User Manual* for details on using module functionality.



Workflow Tabs

Functionality associated with each workflow tab is described below. Note that tapping the **Acquire** or **Data** tab launches a series of sequential screens that guide you through a process, while options in the **Manage** tab may be individually selected at any time to perform distinct tasks.

Home Tab: Displays the instrument and Matrix software logos.

Powered By Matrix™

Also contains the **About Matrix™** button which displays software version details and Support contacts, as well as the **Generate Diagnostic Report**/**Clear All Logs** buttons used to assist Support with troubleshooting technical issues. See *Viewing Software Version* on page *24* and *Generating Diagnostic Reports* on page *50*.

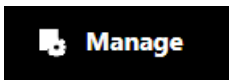
Acquire Tab: Selects the *Data Acquisition* workflow. In the **Acquire** tab you can either select a favorite (with all assay settings pre-defined) or enter *General* and *Consumable Details* information to select an assay before advancing to preview the sample and confirm focus/ fluorescent exposure for each channel. See *Performing a Cell Count* starting on page *31* for more information.

Note: The **Skip Preview** field selection is used to toggle the display of the **Preview** (i.e., **Skip Preview** is set to *No*) and **Count** (i.e., **Skip Preview** is set to *Yes*) buttons.

Data Tab: Selects the *Data Analysis* workflow. You must first select a scan result to view its count results. You can then fine-tune assay parameter settings to perform a recount and create a new scan result. See *Analyzing Scan Results* starting on page *37* for more information.

Use the **Select Result** button 

located in bottom left corner of the screen to return to the previous screen in the process. *Tapping the **Data** tab while already in the workflow no longer returns to the previous screen as it did in earlier releases*.



Manage Tab: Contains the various system lists of favorites, assays, cell types, ACS templates and report templates used in the *Data Acquisition* and *Data Analysis* workflows.

- In the *Favorites* screen you can view the library of favorites available in your system, import or export favorites, manage the list of favorites, as well as create new favorites or modify existing favorite details.

  Favorites enable users to select previously saved counting parameter settings and instantly run samples without having to choose an application, thus bypassing the setup process and possibly the Preview screen (if *Skip Preview* feature is enabled). Scans that are performed frequently can be run with minimal software interaction.

- In the *Assays* screen you can view the library of assays available in your system, import or export assays, manage the list of assays, as well as create new assays or modify existing assay details.

- In the *Cell Types* screen you can view the library of cell types available in your system, import or export cell types, manage the list of cell types, as well as create new cell types or modify existing cell type details.

- In the *ACS Templates* screen you can view the library of ACS templates available in your system, import or export ACS templates, and manage the list of ACS templates. *ACS templates cannot be created or viewed in the Matrix software.*

  ACS Templates can be used for populating data into specified formats when files are generated on export. If the *Auto Open* export option is also selected, the De Novo Software FCS Express application (if installed on your system) is launched for viewing the output file.

- In the *Report Templates* screen you can view the library of report templates available in your system, import or export report templates, manage the list of report templates, as well as create new report templates or modify existing report template details.

See the *Cellometer Matrix Software User Manual* for details on using the **Manage** tab options.

Simplified Workflow

Simplified workflow features have been integrated into the Matrix software graphical user interface (GUI) to streamline user input in the most common data acquisition and analysis workflows.

USER FAVORITES

A key feature introduced in the v6.0 release is the creation of user favorites. A *Favorite* is a collection of count parameters such as an assay, consumable name/type, and reports/exports settings that can be saved for quick re-use. Users can assign images to be associated with favorites for quick reference, specify a prefix to be used for a series of consumables within an experiment, and enable the option of skipping the Preview screen (in favor of proceeding directly to the Count screen).

Favorites enable users to select previously saved counting parameter settings and instantly run samples without having to choose an application, thus bypassing the setup process and possibly the Preview screen (if *Skip Preview* feature is enabled). Scans that are performed frequently can be run with minimal software interaction.

Favorite Selection Panel: The *Favorite Selection* panel is displayed across the top of the Setup screen and highlights either the last used favorite or the system default. To run the selected favorite, simply tap the **Preview** (if enabled) or **Count** button to proceed.

To run a different assay, users can either scroll across the panel to select another favorite or create a new setup by selecting an assay from the dropdown and/or entering new parameter settings.

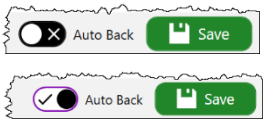
STREAMLINED USER EXPERIENCE

Additional simplified workflow features built into the Matrix software may be used to streamline the overall user experience.

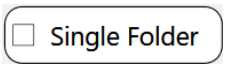
Add Timestamp Button: When entering setup details, tapping the **Add Timestamp** button automatically appends a timestamp to the Consumable ID when the scan result is created. *This button is enabled by default.*

Skip Preview Feature: When entering setup details, the **Skip Preview** field selection is used to toggle the display of the **Preview** (i.e., **Skip Preview** is set to **No**) and **Count** (i.e., **Skip Preview** is set to **Yes**) buttons.

Auto Back Button: If enabled, the **Auto Back** feature toggles functionality of the **Save** button to function as **Save and Back**, automatically returning users to the previous screen when tapped. Tap the **Auto Back** button  (located to left of **Save** button) to enable or disable this feature.



Single Folder Button: When entering setup details in the *Reports and Exports* area, the **Single Folder** button toggles between exporting files using the hierarchical folder structure traditionally available in earlier Matrix releases (button is *not* selected) or to a single folder (i.e., button is selected). *This button is also available when exporting scan results and is disabled by default.*



Back Button: While using the **Acquire** workflow tab, the Matrix software launches a series of screens that guide you through a sequential process. Use the **Back** button located in bottom left corner of the screen to return to the previous screen in the process. *Tapping the **Acquire** tab while already in the workflow no longer returns to the previous screen as it did in earlier releases.*

Select Result Button: While using the **Data** workflow tab, the Matrix software launches a series of screens that guide you through a sequential process. Use the **Select Result** button located in bottom left corner of the screen to return to the previous screen in the process. *Tapping the **Data** tab while already in the workflow no longer returns to the previous screen as it did in earlier releases*.

Focus Methods

Matrix software offers the following two focus methods for the Cellometer Ascend.

IMAGE-BASED AUTO FOCUS

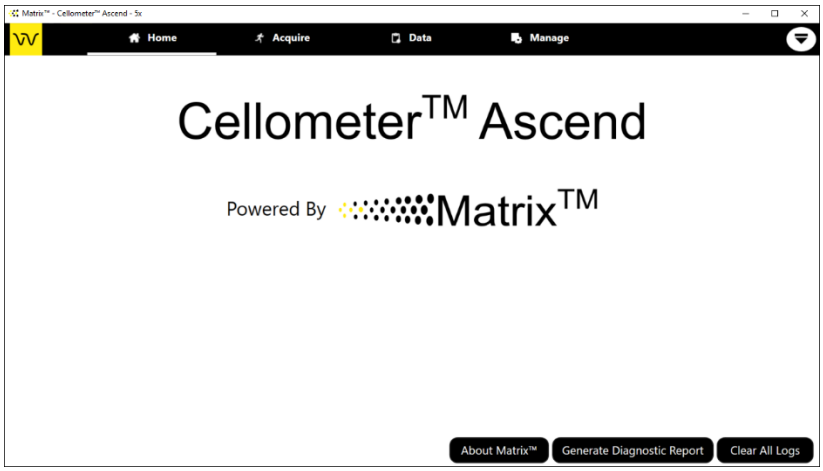
The image-based auto focusing operation relies on the contrast within an image. *Z*-stacking is performed on the initial image, then the software analyzes objects in the image, selects images with the best contrast, and applies an algorithm to sharpen the focus further. Users have the option to perform image-based auto focusing for either the brightfield or fluorescent channels.

SLIDE-BASED AUTO FOCUS

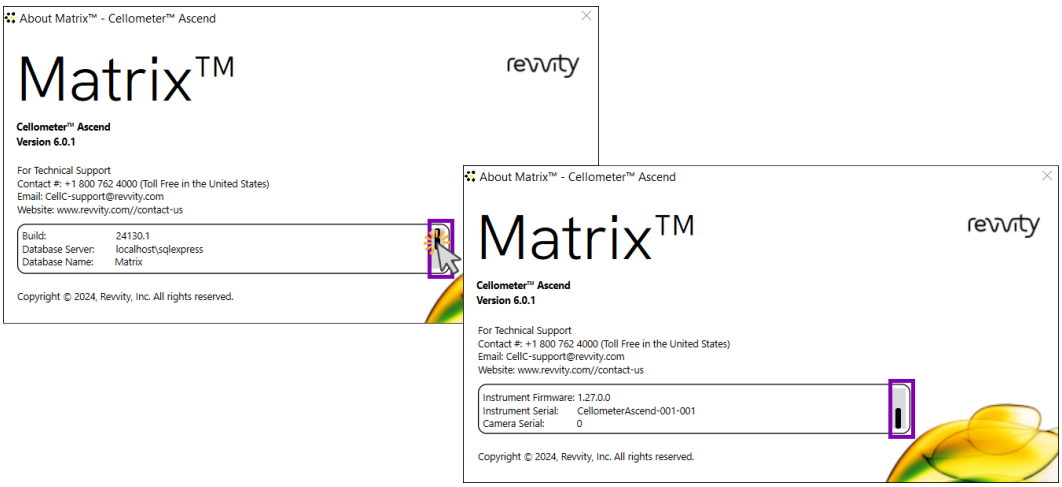
Available for the Cellometer Ascend for the 3-Chamber Slide Only. The Slide-based auto focusing operation allows the software/instrument to focus on the consumable (without needing to focus directly on the sample) by using a target manufactured on the slide.

VIEWING SOFTWARE VERSION

The **Home** tab displays the logo for the instrument to which you are connected and the Matrix software logo. It also contains the **About Matrix™**, **Generate Diagnostic Report**, and **Clear All Logs** buttons.



Tap the **About Matrix™** button to display product information (e.g., instrument type and software version), Support contact information, software details (e.g., build number, database server/name), and instrument details (e.g., instrument firmware version and instrument serial number/camera serial number). *Users may need to tap and drag the scroll bar to view all information*.



For details on using the **Generate Diagnostic Report** and **Clear All Logs** buttons used to assist the Support team with troubleshooting technical issues, see *Generating Diagnostic Reports* on page *50*.

WARNING: Logs may be required to maintain a historical archive. As using the Clear All Logs button will *permanently* remove accumulated logs for the installed Matrix software version, it is recommended that you contact IT *before* clearing logs from your system.

Chapter 6. Viability Methods

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

EVALUATING VIABILITY METHODS

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

Note: Cell concentrations of 2.0 x 10^4 – 2.0 x 10^7 cells/mL can be analyzed on Cellometer Ascend, with a concentration of 2.0 x 10^6 cells/mL being optimal.

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

Dilution or concentration of a cell sample may be required based on the initial concentration. *It is recommended to use cell culture media for dilution.*

Using Trypan Blue Viability Method

Brightfield imaging and the *Trypan Blue Viability* can be used to determine the number, concentration and percentage of live cells for cell lines and cultured primary cells. Brightfield imaging with trypan blue staining is *not* recommended for samples containing debris, platelets or red blood cells. For accurately differentiating nucleated cells, fluorescence is required.

PREPARING A CELL SAMPLE FOR TRYPAN BLUE VIABILITY DETERMINATION

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

For viability measurement, stain cells by combining 20 µL of cell sample with 20 µL of a 0.2% trypan blue staining solution (for a final concentration of 0.1% trypan blue). Gently mix by pipetting up and down 10x.

TRYPAN BLUE STAINING SOLUTION GUIDELINES

Use the following trypan blue staining solution guidelines when preparing cell samples for analysis.

Stain Type	*Use with Cell Sample*	*Dilution Factor*
Trypan Blue (0.2%)	1:1	2

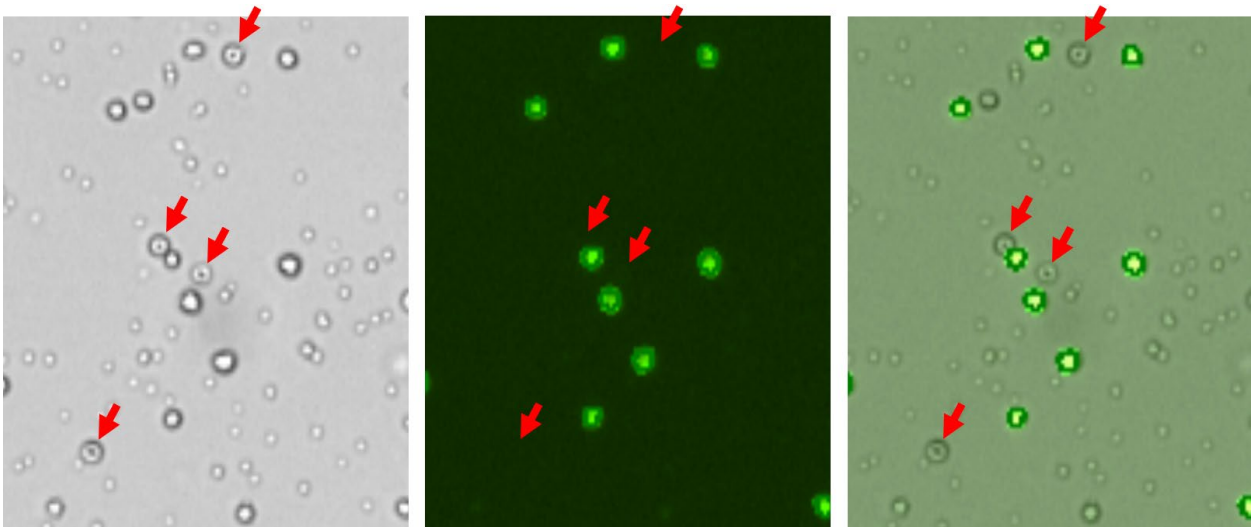
Using AO/PI Viability Method

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

In the *AO/PI Viability Method*, acridine orange (AO) enters all cells and stains their DNA causing nucleated cells to fluoresce *Green* (*470/534 Channel*), while propidium iodide (PI) only enters dead cells with compromised membranes and stains their DNA causing them to fluoresce *Red* (*531/655 Channel*).

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

As a result, debris and non-nucleated cells do not interfere with nucleated cell counts when using the AO/PI viability method.



In images captured by Cellometer Ascend using the AO/PI viability method, red blood cells (RBCs, marked by red arrows) seen in the brightfield image (on left) are *not* seen in the fluorescent image (in middle), but are clearly identified in the overlay image (on right). Only nucleated cells are counted using the AO/PI staining method resulting in a more accurate total cell count and percent viability calculation.

PREPARING A CELL SAMPLE FOR AO/PI VIABILITY DETERMINATION

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

For viability measurement, stain cells by combining 20 µL of sample with 20 µL of AO/PI staining solution. For whole blood and other viscous samples, draw sample in and out of the pipette tip at least once prior to transferring for staining. Gently mix stained cell solution by pipetting up and down 10x before adding sample to counting chamber.

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

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

AO/PI STAINING SOLUTION GUIDELINES

Use the following AO/PI staining solution guidelines when preparing cell samples for analysis.

Stain Type	*Use with Cell Sample*	*Dilution Factor*
AO (CS1-0108-5mL)	1:1	2
PI (CS1-0109-5mL)	1:1	2
AO/PI (CS2-0106-5mL)	1:1	2
AO/PI (CS2-0106-25mL)	1:1	2

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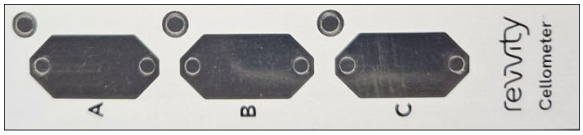
Chapter 7. Sample Preparation

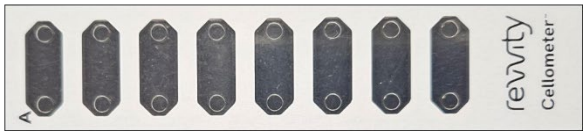
This chapter describes preparing Cellometer counting chamber slides and loading counting beads/cell samples into counting chambers.

PREPARING COUNTING CHAMBER SLIDES

1. For *3-Chamber* and *8-Chamber* slides, remove protective film from both sides of the slide. Do *not* touch or write on the clear optical window areas of the slide.

   Note: It may be difficult to peel protective film from the slide. One method is to adhere tape to the top of film and then pull tape off (with film attached).





2. Place the Cellometer disposable counting chamber slide on a fresh Kimwipe.
3. To prepare multiple samples on the same slide, label individual counting chambers (e.g., *#1*, *#2, etc.)* in the white margin of each chamber. Be careful to *not* touch the clear optical window of the counting chamber. *Note that Ascend 3-Chamber slides have already been labeled with A, B, and C.*

LOADING SAMPLES INTO COUNTING CHAMBERS

Cellometer Ascend *3-Chamber* and *8-Chamber* consumable slides consist of independent chambers manufactured to a precisely controlled height. A cell suspension of 20 µL is pipetted into a chamber through its inlet port.

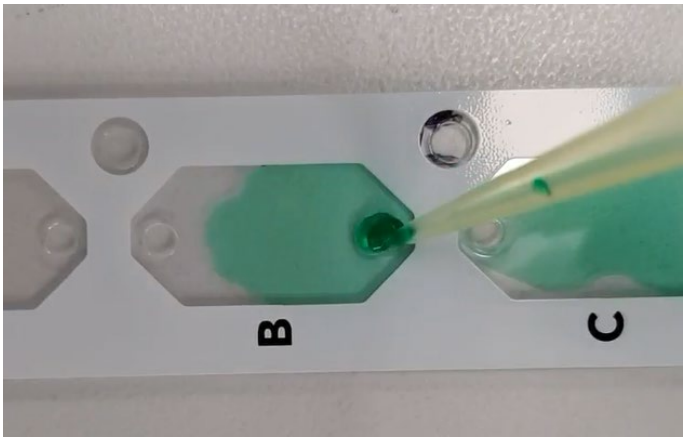
1. To prepare the sample for counting, invert the tube containing your sample 10 times, then pipette up and down 10 times. This will help to evenly suspend cells. Do *not* shake sample as it may damage cell membranes.

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

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

2. Immediately transfer the recommended volume of sample into a counting chamber by pipetting directly onto its inlet port. For 3-Chamber slides, load 20 µL per chamber. For 8-Chamber slides, load 10 µL per chamber.
3. When handling the slide loaded with sample, be careful to *not* touch the clear optical windows.

Load a counting chamber by touching the pipette tip to the chamber's inlet port and slowly pipetting the entire recommended sample volume per chamber all at once. Capillary force automatically spreads the sample within the chamber. Each counting chamber can hold a separate sample as there is no mixing between the two individual chambers on a slide.



Note: Ascend 8-Chamber slides are compatible for use with a standard multi-channel pipette.

Chapter 8. Matrix Counting and Analysis Workflow

This chapter presents the basic workflow for counting cells using the Cellometer Ascend powered by Matrix software, including best practices and tips.

For a complete reference on using Matrix software, see the *Cellometer Matrix Software User Manual* for details.

PERFORMING A COUNT

If you are using a *Favorite* containing pre-defined parameter settings, performing a count can be as simple as selecting that favorite and inserting the prepared sample slide. Favorites enable users to select previously saved counting parameter settings and instantly run samples without having to choose an application, thus bypassing the setup process and the Preview screen (if *Skip Preview* feature is enabled). Scans that are performed frequently can be run with minimal software interaction.

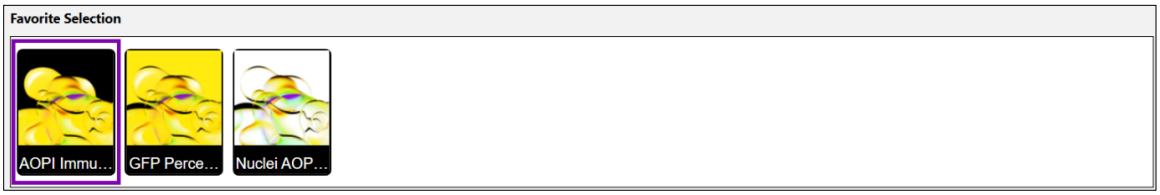
To customize count parameters before performing a count, choose an assay and enter parameter settings, load the samples, and then preview the samples (if *Skip Preview* feature is enabled) before tapping the **Count** button.

Choosing Data Acquisition Workflow

1. In the Navigation Bar, tap the **Acquire** tab (if not already displayed by default) to display the Setup screen.
2. Either select a favorite from the **Favorite Selection** panel (see *Selecting a Favorite* on page *31*) or choose an assay and customize parameter settings for a count in the *General*, *Consumable Details*, *Well Names*, and *Reports and Exports* areas (as described in *Entering Parameter Settings* on page *32*).

SELECTING A FAVORITE

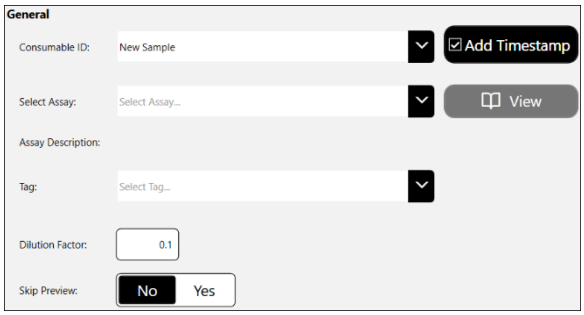
The *Favorite Selection* panel is displayed across the top of the Setup screen and highlights either the last used favorite or the system default. Scroll across the panel to select a favorite.



To run the selected favorite, simply tap the **Preview** button (if enabled; skip to *Previewing Samples* on page *33*) or the **Count** button (see *Tapping the Count Button* on page *36*) to proceed.

To run an assay available in the dropdown, users can customize parameter settings before performing a count.

ENTERING PARAMETER SETTINGS

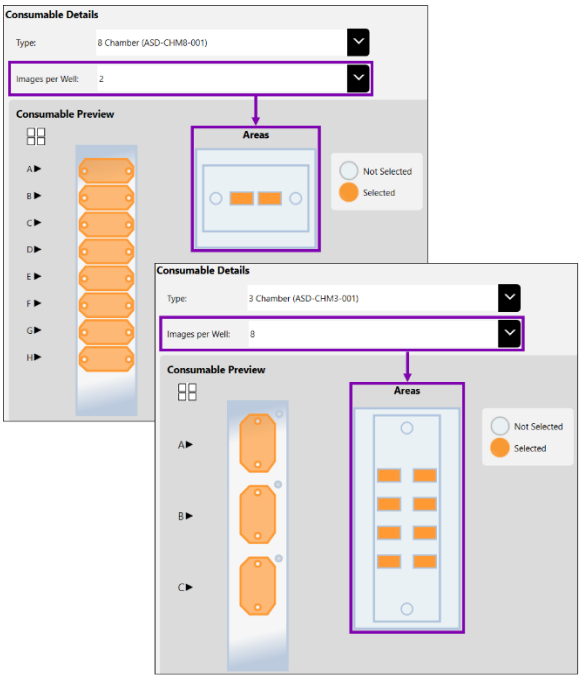


1. In the *General* area, enter a **Consumable ID**. *If a consumable ID is not entered, a date/time stamp will be appended to the "New Sample" default (e.g., New Sample 2024/03/25-10:58:09).*
2. Use the **Select Assay** dropdown to choose an assay and confirm the assay description displayed.

   To view and/or edit assay details, tap the **View** button. Tap **Back** (if no changes were made) or **Save** (to save any changes) to return to the Setup screen.

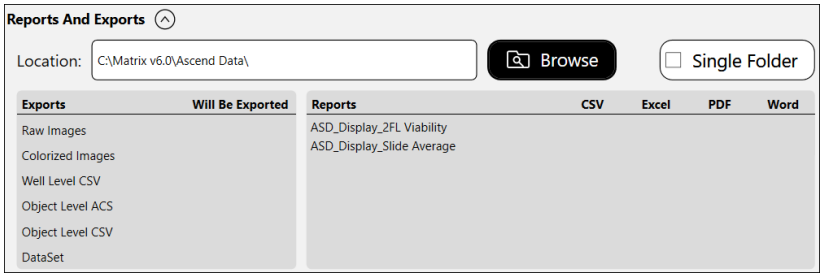
   Note: If an assay is locked for editing (i.e., the **Category** field shows *Locked* as enabled instead of *Unlocked*), users must first tap **Save As** to save it as a new assay before they can edit its parameters.
3. If desired, add a **Tag** (e.g., to create a time course series for use in custom reporting). In addition, you can change the value in the **Dilution Factor** field to indicate the final dilution factor for the sample.
4. In the **Skip Preview** field, select **No** to enable the **Preview** button or **Yes** to skip previewing the sample and proceed directly to performing the count.
5. In the *Consumable Details* area, choose consumable **Type** (e.g., *3 Chamber* or *8 Chamber*).
6. Select number of **Images Per Well**. *For 3 Chamber slides, available values are 2, 4, and 8.*

   In the *Consumable Preview* area, the visualization of a chamber containing the selected number of *Images Per Well* (i.e., representing cross-sections of the counting chamber area) to be taken during the scan is depicted.



SETTING AUTO EXPORT LOCATION

Expand the *Reports and Exports* area to identify a **Location** for automatically saving scan results. Tap the **Browse** button and navigate to a folder on your Operating Computer or network to define the default export path. *This default path will remain the same within the software until it is manually changed.*



In addition, *Exports* (see descriptions provided below) and output files for *Reports* (e.g., *CSV*, *Excel*, *PDF*, and *Word*) defined for the current assay are displayed.

Sample *Exports* include:

Raw Images	Represents *Black and White* high-resolution PNG images for each channel in the assay.
Colorized Images	Represents colorized, high-resolution PNG images of all acquired channels. *A single colorized image represents individual channel images superimposed one on top of another.*
Well Level CSV	Represents well-level data including well name, calculation run, channel, count, mean size, etc. for each selected well in Comma Separated Values (CSV) format.
Object Level ACS	Represents object-level data for each well including type, classification, size, circularity, area, perimeter, etc. in Image Cytometry Experiment (ICE) format. *Exported data may be associated with an ACS template and automatically opened for viewing in De Novo Software FCS Express (if specified in auto export options for the assay).*
Object Level CSV	Represents object-level data for each well including type, classification, size, circularity, area, perimeter, etc. in Comma Separated Values (CSV) format.
Data Set	Represents a database file containing all images, results, assays, cell types and report templates associated with the scan result. *A data set is required to reload data for analysis or if you need help from Support to optimize assay and/or cell type parameters.*

To manage *Exports* and *Reports* defined for the selected assay, tap the **View** button and expand the *Reports and Exports* option. Select *Exports* by tapping on file type buttons and manage *Reports* as necessary. Changes to the assay can either be saved to the current assay (i.e., any changes made will also be applied to other scan results that use the assay) or saved as a copy with a new name. Edited assays can be used for data acquisition. See the *Cellometer Matrix Software User Manual* for details on editing an assay to manage exporting and reporting options.

Loading Samples

If you have already prepared and loaded a consumable counting slide with samples using instructions from Preparing Counting Chamber Slides starting on page 29, skip to Previewing Samples, below.

1. Power on the instrument.
2. Remove the protective film from both sides of the slide.
3. Slowly pipette 20 µL of cell/bead sample directly into one or more inlet ports of slide chambers.

   Note: If testing for cell viability, you must stain the sample before loading it into the counting chamber. See *Trypan Blue Staining Solution Guidelines* on page *25* or *AO/PI Staining Solution Guidelines* on page *27*.

4. Pick up the slide loaded with cells/beads (being careful to *not* touch the clear optical windows) and insert into the Sample Slot until it is met with resistance. The slide automatically retracts into the instrument.

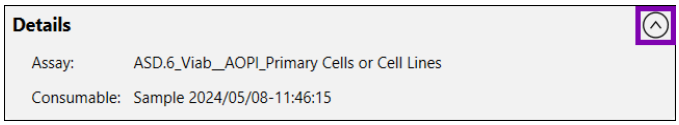
Preview Mode

If *Preview* mode is enabled (i.e., **Skip Preview** field in Setup screen is set to **No**), the instrument engages its camera for viewing samples and displays the Preview screen. Users can view live images of samples in selected wells, preview available channels for *Imaging Mode* associated with the assay, adjust instrument focus and confirm fluorescent exposure for each channel.

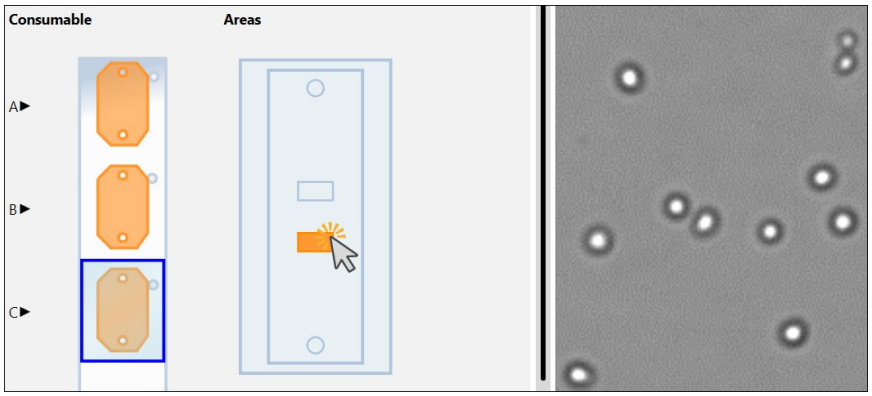
Note: If the **Skip Preview** field in Setup screen is set to **Yes**, the software skips previewing samples and goes directly to *Count* mode. See *Count Mode* on page *36* for details.

PREVIEWING LIVE IMAGES

Expand the *Details* area at the top of the Prevew screen to view assay and consumable ID.



In the *Areas* visualization of a slide counting chamber, tap on different areas (i.e., representing cross-sections of the counting chamber or "well"). As you move from area to area, the live image changes per your selection.

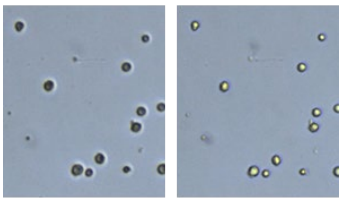


To zoom in/out of an image, move the mouse to hover cursor over the viewing pane and turn the scroll wheel or, if using the touchscreen, apply universal gestures (e.g., touch the center of the image with two fingers and then slowly spread them apart to zoom in and reverse this action to zoom out.) To move a zoomed image around, tap and drag the image to a new location as needed.

ADJUSTING FOCUS

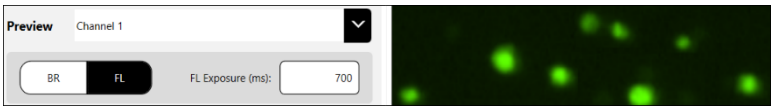
To adjust focus of the live image being previewed, tap the **Auto Focus** button. *Pinch to zoom the image, if needed.*

Obtaining good focus is key to ensuring accurate cell counts. Once good focus has been achieved, the instrument should perform most counting operations with only minor adjustments.



PREVIEWING CHANNEL IMAGES

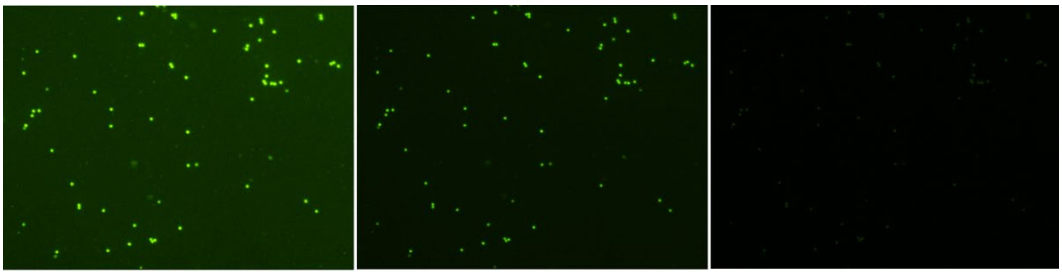
In the *Preview* area, channels available for viewing (e.g., *Channel 1* and *Channel 2*) are based on the assay Imaging Mode. The *Channel 1*/*BR* image is displayed by default. Tapping the **FL** button displays the *Channel 1*/*FL* image.



Note: If only one channel is available, the *Preview* field channel dropdown will be hidden.

To view images for another channel, select it from the **Preview** field dropdown. Tap the **FL** button to view fluorescence in that channel. A sample *Channel 2*|*FL* image is shown below.

When previewing fluorescent images, confirm that FL signal is strong but has a low, dark background. Modify the **FL Exposure** to increase (prolong) or decrease (shorten) the exposure time accordingly.



Notes:

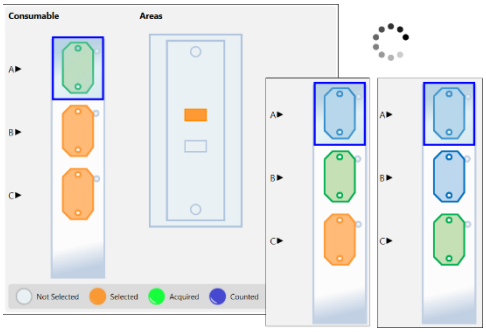
- For BR/FL imaging modes, each channel will be associated with two images – *Brightfield* (*BR*) and *Fluorescent* (*FL*). These images are referred to in assay channel mappings as *BR1*/*FL1* and *BR2*/*FL2*.
- When working with assays that have two channels, use the *Channel 1*/*BR* image to adjust focus and then select the *FL* image to confirm exposure. For *Channel 2,* you only need to select the *FL* image to confirm exposure since focus of its paired *BR* image is adjusted automatically when you performed the task for *Channel 1*.
- If *FL* images are available for both *Channel 1* and *Channel 2*, a slight offset may exist between *BR1* and *BR2* images due to the distance the camera must travel.

Count Mode

Once you have completed previewing the live image for the sample, tap the **Count** button located at the bottom of the Preview screen. The instrument camera acquires sample images as specified by the selected assay which are then used by the Matrix Software to calculate count results according to defined cell type parameters. *Depending on the number of selected wells/images per well and defined assay parameters, the counting process can take a few seconds to up to a few minutes.*

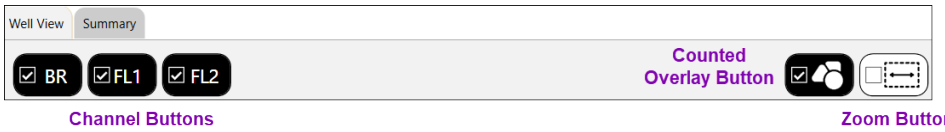
Note: If *Count* mode is enabled (i.e., **Skip Preview** field in Setup screen is set to **Yes**), the instrument skips the Preview screen and immediately starts to perform a *Count*.

As the system acquires sample images and calculates count results, the colors used to mark the *Areas* visualization of a slide counting chamber (where the number of highlighted areas is based on the *Images Per Well* value) will change to indicate image status (i.e., from *Selected* to *Acquired* to *Counted*) as shown in the legend displayed below the Well Map.



You can tap on an area as soon as it is counted to display results below the viewing pane. *Count results will be displayed, printed and exported based on report templates defined for the assay.*

Well images displayed can be varied by toggling on/off available *Channel* buttons (available across the top of the viewing area of the **Well View** tab) and enhancing the *Zoom* magnification.

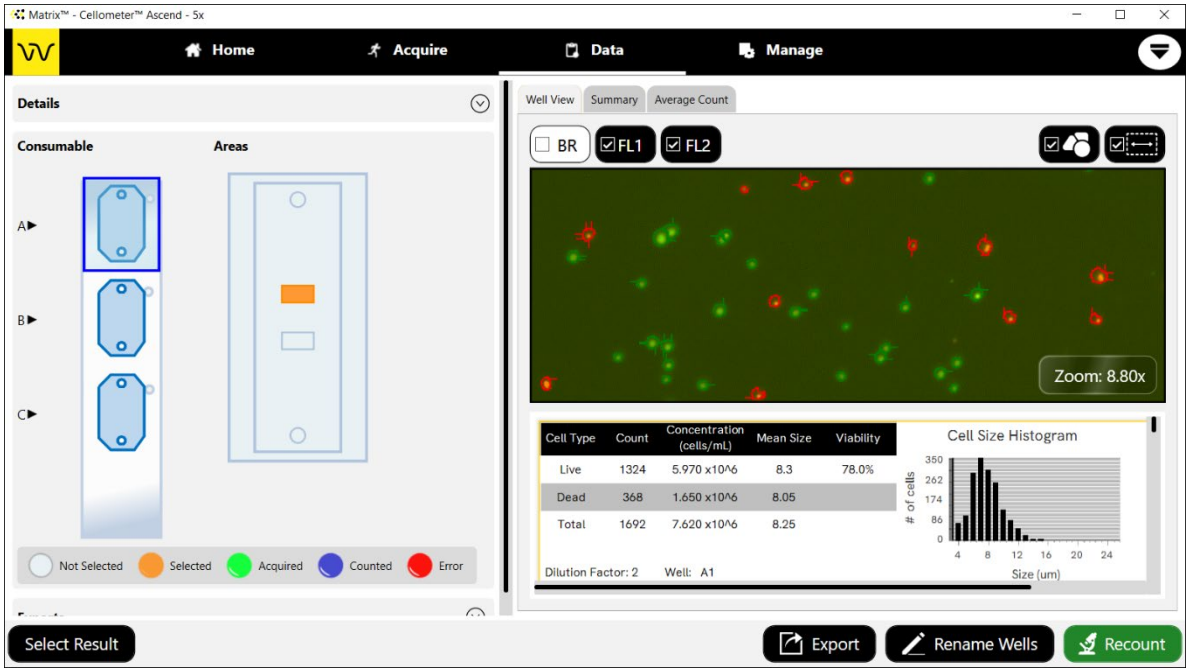


To vary the area of the image displayed, tap inside the viewing pane with a finger or stylus pen, drag and drop the image to a new area, and pinch to zoom (i.e., touch the center of the image with two fingers and then slowly spreading them apart to zoom in and reverse this action to zoom out).

When counting of all wells is complete the scan result will be added to the top of the Results List displayed on the Select screen. In addition, *Reports and Exports* output files defined for the current assay are automatically stored in the specified location.

ANALYZING SCAN RESULTS

When analyzing scan results, an image is displayed in the viewing pane for a specific counting chamber and area.



To vary the image displayed, users can tap different chambers (in the *Consumable* area) and different areas (in the slide visualization), or tap inside the viewing pane to drag and drop the image. Users can also pinch to zoom the image (i.e., touching the center of the image with two fingers and slowly spreading them apart to zoom in and then reversing this action to zoom out).

The **Images Per Well** value (e.g., *2*, *4* or *8*) defined in parameter settings determines the number of independent images of a slide chamber/well available for viewing and export, rather than one large image of the whole chamber, significantly reducing image load time and improving memory handling. During export, the software appends a "*WellNumber_N*" indicator (e.g., *A1_1*, *A1_2*, *A1_3*, *A1_4*) to each filename indicating its position index for the area in the chamber/well.

Understanding Default Report Tabs

Report tabs initially displayed across the top of the viewing pane are associated with report templates that have been assigned to the current assay and are enabled by default to format the presentation of scan result data.

Although the **Well View** tab will always be enabled, you can change the report template used for its display. In addition, you can disable default report tabs, change current report template assignments and add new tabs to meet your reporting needs. See the *Cellometer Matrix Software User Manual* for custom reporting details.

Changing Well View Image Display

The **Well View** tab will always be displayed in the Results screen and includes both an image and the associated count results for the selected "well" or chamber. *If multiple images were taken (based on your Images Per Well selection), they will each represent a separate area in the chamber (e.g., 4 Images Per Well will result in an image of each quadrant).* Tap and drag the image to view other locations (or areas) in the chamber. To display images for other acquired samples, select other highlighted areas in the Well Map.

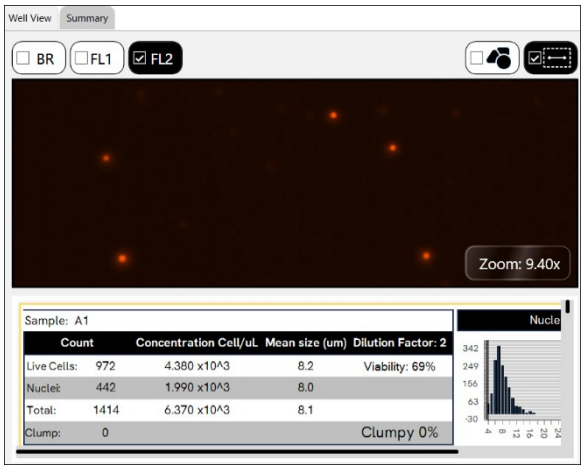
To zoom in/out of the image, move mouse to hover cursor over the viewing pane and turn the scroll wheel or, if using a touchscreen, by applying universal gestures (e.g., touching the center of the image with two fingers and then slowly spreading them apart to zoom in and reverse this action to zoom out). If **Zoom** button is enabled, *Zoom* magnification is displayed in bottom right corner of the viewing pane and can be increased up to 10.00x.

At the bottom of the **Well View** tab is a report containing "well-" or chamber-level details for the sample. *If an **Images Per Well** value was selected, the report represents consolidated data from all images.*

Varying Well View Channels/Counted Overlay Display

In the **Well View** report tab displayed by default, the following buttons may be available across the top of the viewing pane based on the Imaging Mode defined in the current assay.

- Tap the *Brightfield* (**BR)** or *Fluorescence* (e.g., **FL1** or **FL2**) buttons to select/de-select channels used in the image display. *Channel views are overlaid on top of each other.*
- Tap the **Counted Overlay** button / to show/hide the graphic overlay that identifies *Counted* cells by surrounding them with color-coded outlines. *For 2-channel Viability assays, Green is used for counted/live cells, Red for dead cells and Yellow for cells not counted (e.g., if larger than the specified cell diameter).*
- Tap the **Zoom** button / to enable/disable display of current *Zoom* magnification in bottom right corner of viewing pane. *Zoom feature will still be functional even if not displayed*.

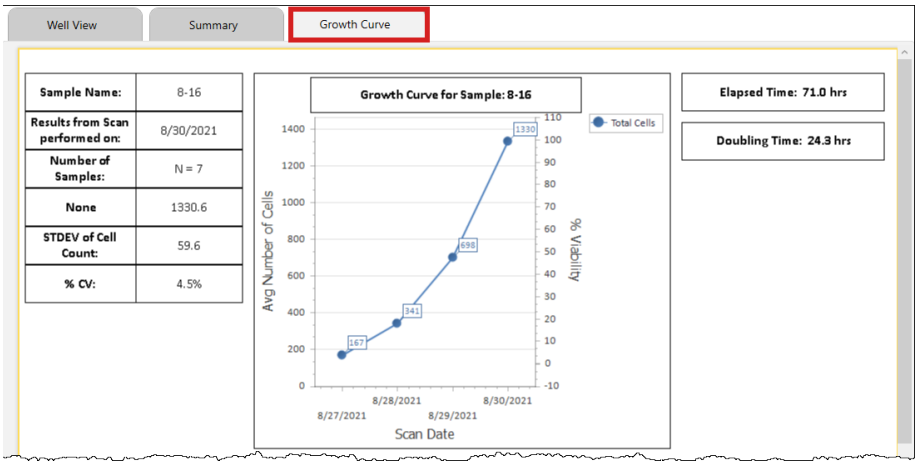


Understanding Custom Reporting

The Matrix custom reporting feature allows you to assign and/or modify report templates to be used by assays when generating scan results. You can change the report template used for display of the **Well View** report (which is always displayed) and add report templates to be enabled as tabs or output as specific file types (e.g., *CSV*, *Excel*, *PDF* and *Word*) that can be opened/printed upon report generation.

Note: Any changes made to *Reporting* options for an assay will be saved with the assay and applied to all other scan results using that assay the next time a recount is performed.

For example, a custom **Growth Curve** report tab has been enabled in the scan result shown below. Tapping this tab displays data in the format defined by the associated report template (i.e., illustrating calculation of a growth curve for a time course series of scan results).

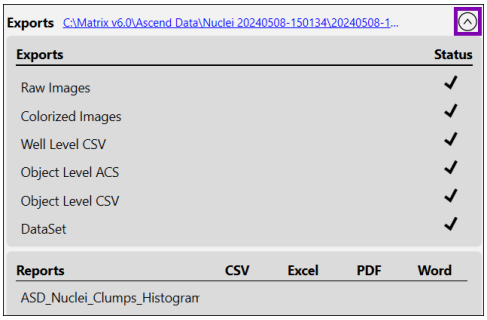


See the *Cellometer Matrix Software User Manual* for custom reporting details.

EXPORTING/PRINTING SCAN RESULT DATA

Verifying Auto Export

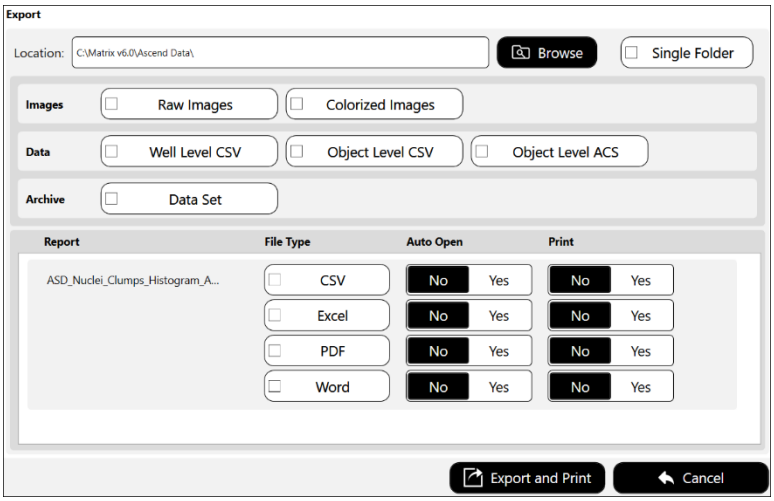
Expand the *Exports* area in the Results screen to verify automatic exports were completed as defined for a favorite or assay and tap the **Exports** location link to open the folder where exported scan result files are stored.



In addition, if you defined any output file types (e.g., *CSV*, *Excel*, *PDF* and *Word*) to be generated for assay report templates, you can verify that selected file type exports were also completed.

Exporting and Printing Scan Results (Manual Settings)

To manually select additional export options (images/data) for scan result files as well as generated output files for reports, tap the **Export** button located at the bottom of the Results screen. The Export dialog is displayed.



Confirm the export **Location** and if necessary update this path by tapping the **Browse** button, navigating to a folder on your Operating Computer or network, and tapping **OK**.

Select scan result **Images** (e.g., *Raw Images* or *Colorized Images*), **Data** (e.g., *Well Level CSV*, *Object Level CSV* and *Object Level ACS)* and **Archive** (e.g., *Data Set*) file options to be exported to the specified location. *Data Sets are stored as .SCANRESULT files.*

In addition, for reports associated with the scan result you can select generated output file types (e.g., *CSV*, *Excel*, *PDF* and *Word*) to be exported and indicate if files are to be opened automatically and/or printed upon export.

Note: If the *Object Level ACS* option is selected, the screen expands to display if an ACS template has been assigned to the assay and offers an *Auto Open* option after the report is generated. *If no ACS template is displayed, users can still export object level ACS data assuming they have the export privilege.* To change the ACS template assigned to an assay, users will need to edit the assay.

For report templates associated with the scan result you can select generated output file types (e.g., *CSV*, *Excel*, *PDF* and *Word*) to be exported and indicate if files are to be opened automatically and/or printed upon export.

Note: Any changes to export options are applied only when manually exporting scan results (i.e., by tapping the **Export and Print** button) and will *not* be saved with the assay or scan result.

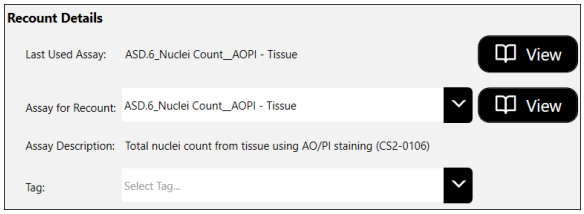
PERFORMING A RECOUNT

If you find it necessary to fine-tune assay parameters after reviewing your data results, tap the **Recount** button located at the bottom of the Results screen. The Recount screen is displayed.

Once you have made any necessary changes (per the options described below), tap the **Recount** button located at the bottom of the screen. After a recount is performed, the Navigation Bar returns to the Results screen.

Refining Assay Details/Selecting New Assay

In the *Recount Details* area you can view parameter settings for the *Last Used* assay, select a new assay from the dropdown or view the current assay to edit parameter settings to be used for the recount.

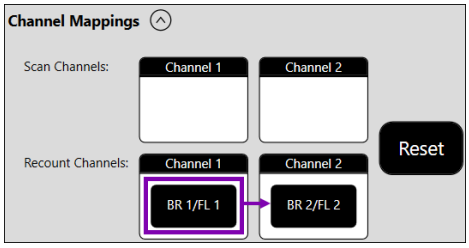


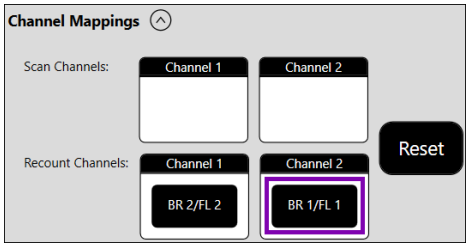
Managing Channel Mappings

Expand the *Channel Mappings* area to identify *Scan Channels* and *Recount Channels* for the Imaging Mode associated with the current assay. Performing a recount uses channel mappings as defined by default (i.e., *Recount Channels* will remain in same positions used for the original *Scan Channels*).

Note: Each channel will have separate brightfield and fluorescent images as depicted in the mapping indicators (i.e., *BR1/FL1* and *BR2/FL2*).

To manage channel mappings for use in a recount, tap on a mapping indicator and drag it to a new channel. The mapping indicator in that channel will swap positions with the one you are dragging automatically.

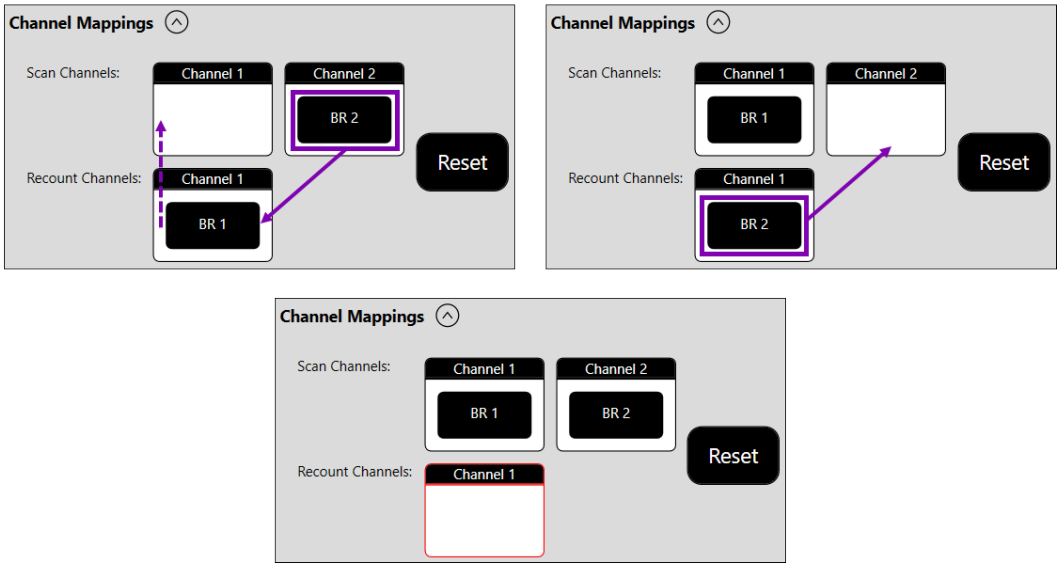




To return channel mappings to the positions they were in when you first expanded the *Channel Mappings* area, tap the **Reset** button followed by **Yes** to confirm the action.

If a new assay selected for recount has fewer channels or you edit the assay previously used to reduce the number of channels, mappings displayed are updated to reflect available channels. To manage channel mappings for use in the recount, tap on mapping indicators and drag them to an available position in the *Recount Channels* area.

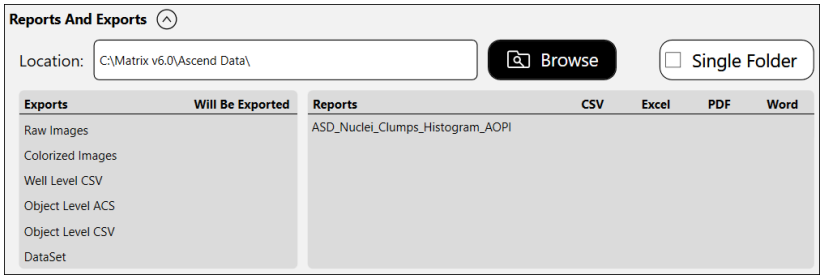
2 Channel Assay Reduced to 1 Channel for Recount



If you drag indicators from *Recount Channels* to their home *Scan Channels* locations, a red outline is used to highlight empty *Recount Channels* locations. All *Recount Channels* must contain an indicator prior to recount.

Modifying Auto Export Options

Expand the *Reports and Exports* area to identify a **Location** for automatic exports of images/data and generated output files for reports. Tap the **Browse** button and navigate to a folder on your Operating Computer or network to define the default export path. *This path will remain as the default in the software until it is manually changed.*



In addition, *Exports* (e.g., *Raw Images*, *Colorized Images*, *Well Level CSV*, *Object Level ACS*, *Object Level CSV* and *Dataset*) and output file types for *Reports* (e.g., *CSV*, *Excel*, *PDF* and *Word*) defined for the assay will be indicated with a blue checkmark.

Note: To modify *Report and Export* settings displayed, tap the **View** button for the selected assay in *Setup Details* area and expand the *Reports and Exports* section to update *Exports* and *Reports* file types selected.

Tapping the Recount Button

Once you have completed entering recount details for the samples, tap the **Recount** button located at the bottom of the Recount screen. The Matrix software performs a recount of the scan using the modified parameters and displays the new scan result.

BEST PRACTICES AND WORKFLOW TIPS

- The *Images Per Well* field in the Setup screen allows users to specify the number of images to be taken of the counting chamber/well. Choosing the lowest number of images (e.g., *2)* provides a high-level overview of data and provides the fastest counting speed. Choosing *4* or *8* images increases the amount of data collected by partitioning the sample into sections and averaging the results from all images, thus improving the precision of calculations for statistical analysis but requiring more time to obtain count results. *Regardless of the number of Images Per Well selected, count results are for the entire counting chamber or "well" (i.e., representing an average of the results).*

- While using the **Acquire** workflow tab, the Matrix software launches a series of screens that guide you through a sequential process. Use the **Back** button 

located in bottom left corner of the screen to return to the previous screen in the process. *Tapping the **Acquire** tab while in the workflow no longer returns to the previous screen as it did in earlier releases.*

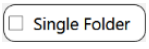
- While using the **Data** workflow tab, use the **Select Result** button 

to review other count results *after* viewing the currently selected scan. *Note that when a scan result is created for a sample as the result of a count, it is displayed at the top of the Results List.* Double-tap on any scan result in this list to view it. See the *Cellometer Matrix Software User Manual* for details on managing scan results contained in the *Results List*. *Tapping the **Data** tab while in the workflow no longer returns to the previous screen as it did in earlier releases*.

- When viewing scan results, if you find it necessary to fine-tune assay and/or cell type parameter settings and re-analyze the data, tap the **Recount** button at the bottom of the Results screen. You can edit either *Last Used Assay* parameters or select a new *Assay for Recount* and edit its parameters as necessary to meet your requirements. See the *Cellometer Matrix Software User Manual* for details on editing assay and/or cell type parameter settings.

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

- When exporting scan results, the Matrix software creates a hierarchical folder structure (by default) in the export location defined for the scan result – a top-level *consumable_ID* folder (where *consumable_ID* represents the name you entered in the Setup screen) and a <*date_time*> subfolder (where *date_time* represents the date/time stamp of scan images). Within this second-level folder are additional folders (i.e., a *consumable_ID* folder containing scan images and a <*date_time*> folder containing the initial count results). *Contents of this <date_time> subfolder will vary based on selected export options.* Each time you perform a recount on the scan images, a new <date_time> subfolder is created for the results.

  When entering setup details in the *Reports and Exports* area, users can select the **Single Folder** button 

which toggles between exporting files using the hierarchical folder structure described above (button is *not* selected) or to a single folder (i.e., button is selected). *This button is also available when exporting scan results. This button is disabled by default.*

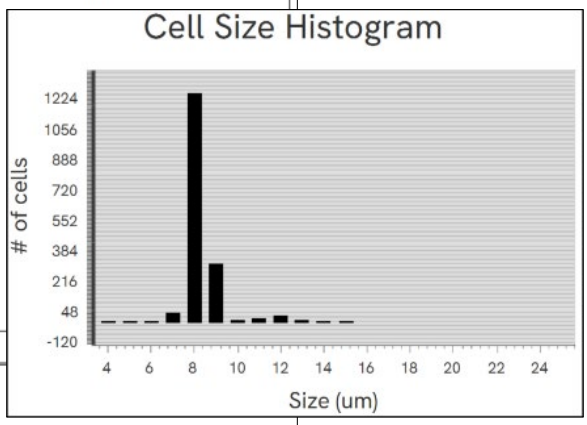
- Data analysis features such as a *Cell Size Histogram* and *Sample Adjustment Calculator* can easily be built into report templates to facilitate and enhance results visualization and evaluation. See the *Cellometer Matrix Software User Manual* for details on editing report templates and how to add them as tabs to customize assay scan results.

Count	Concentration (cells/mL) at Dilution Factor: 1	Mean Size (um)	
Live: 599	0.664 x10^6	8.5	Viability: 54.0 %
Dead: 515	0.576 x10^6	8.9	
Total: 1114	1.240 x10^6	8.9	

Sample Calculator

Target Live Concentration (cells/mL)	Add (x)mL of diluent to 1mL of cell sample	Spin down sample, remove supernatant and resuspend pellet in (x)mL of diluent
100,000	5.34	
200,000	2.17	
250,000	1.54	
500,000	.27	
750,000		.85
1,000,000		.63
1,500,000		.42

Target Number of Live Cells	(x)mL of cell sample needed
100,000	.158
500,000	.789
1,000,000	1.577
1,500,000	2.366
2,000,000	3.155
2,500,000	3.943
5,000,000	7.886
10,000,000	15.773



Sample Name: Jurkat cells
Time Stamp: 4/2/2024 3:00:56 PM
Assay Name: MX.6_Viab__AOPI_Cell Line
Assay Description: Total cell concentration and % viability

Chapter 9. Cleaning, Maintenance and Storage

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

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

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

CAUTION: Allow for flammable agents used for cleaning or disinfecting to completely evaporate before powering the instrument ON.

CLEANING

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

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

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

ROUTINE MAINTENANCE

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

WARNING: Do not remove the instrument cover due to an electric shock hazard. For assistance, contact Support.

Contacting Support

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

Preventive Inspection and Maintenance

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

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

STORAGE

When preparing the instrument for storage:

- Always thoroughly clean the instrument, cables and any of the accessories or consumables before storage.
- Check for any damage and if possible, re-package the Instrument and Operating Computer in the original boxes.
- Ensure that storage temperature and spatial requirements are met (see *Site Preparation* on page *16*).
- DO NOT put anything on the instrument or the box in which it is stored.

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

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

Chapter 10. Troubleshooting and FAQs

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

TROUBLESHOOTING AND INSTRUMENT MESSAGES

Instrument cannot be powered on

- Check to ensure the Power Supply and Power Cord provided with the instrument are being used.
- Check to ensure Power Supply and Power Cord are *not* kinked or tangled.
- Check to ensure Power Supply and Power Cord are plugged in properly to both instrument and electrical outlet accordingly.
- If Power Cord is plugged into a surge protector (*recommended*), be sure that surge protector is powered on.
- Check to ensure the Power Switch is in the *ON* position.

Once the instrument has been powered on, confirm the light on front of the instrument (above *Sample Slot*) is lit.

Instrument displays an error or hangs during image preview/acquisition

If the instrument displays an error or hangs during image preview/acquisition, exit the application by closing the window and then re-launch the software by double-tapping the **Matrix** icon. *If it is necessary to access the Task Manager application, swipe up from the bottom of the touchscreen to display the taskbar.*

If the error persists, power cycle the instrument as indicated below. Launch the software and proceed with image preview/acquisition.

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

Note: If the instrument continues to hang during image preview/acquisition, verify that sufficient storage space is available on the Operating Computer. If local storage is full, use the Matrix software to export any scans that can be kept in an external or server storage system, and then delete as many scans as possible. *If scans are deleted, click the **Recover Free Space** button.*

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 an email to: CellC-support@revvity.com

FREQUENTLY ASKED QUESTIONS

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

Contact Support with the serial number of the instrument to request a replacement power cord/cable.

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

When preparing a sample to test for cell viability, ensure that the stock solution is being used as indicated in *Trypan Blue Staining Solution Guidelines* on page *25* or *AO/PI Staining Solution Guidelines* on page *27*. Using stains in a concentration *higher* than recommended concentrations will make the cells more difficult to detect and may result in counting inaccuracies.

In addition, ensure that:

- The viability method is appropriate for the cell type. See *Evaluating Viability Methods* on page *25* for details.
- The current assay and/or cell type is appropriate for the sample. See the *Cellometer Matrix Software User Manual* for more information about editing assays and cell type parameter settings.

Chapter 11. Revvity 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 and generate diagnostic reports to assist with troubleshooting.

SCOPE OF SUPPORT SERVICES

Revvity provides customers with outstanding support including the following services:

- Online and in-lab customer training
- Creation of new cell types
- Optimization of counting parameters
- Creation of new report templates
- 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, 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 an email to CellC-support@revvity.com

Reporting an Issue to Support

If a technical issue encountered cannot be resolved using troubleshooting steps presented in this guide (see *Troubleshooting and Instrument Messages* on page *47*) or if the issue persists after rebooting the instrument, perform the following steps *before* contacting Support to report the issue:

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.
   See *Device SN Label* on page *11*.

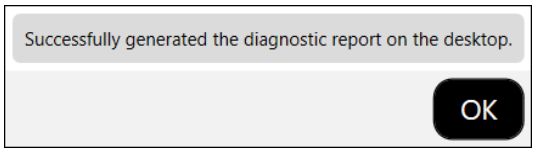
Gathering these details *prior* to contacting Support will be helpful as they troubleshoot the technical issue.

GENERATING DIAGNOSTIC REPORTS

To generate a diagnostic report that can be emailed to Support when experiencing a technical issue:

1. Tap the **Home** tab and then the **About Matrix™** button.

2. Tap the **Generate Diagnostic Report** button followed by **OK** in response to the confirmation prompt.



3. From the desktop, tap the generated ***Diagnostic_YYYYMMDD.zip*** folder (where *YYYYMMDD* represents the date on which file was generated) to display files in the folder.

   Files in the zipped folder include logs (located in *C:\logs\Matrix\vXXX* where *XXX* represents installed version) and configuration files (located in *C:\ProgramData\Revvity\Matrix\vXXX\Configurations*).

4. Attach the zipped folder to an email, include the *Support Ticket ID* (if assigned) in the Subject line and send to: CellC-support@revvity.com

It may be helpful to clear all logs before reproducing the sequence of steps leading up to an issue and/or to reduce the size of the diagnostic report to be sent to Support. *Logs are generated automatically by your system daily. Keep in mind that clearing all logs will remove files accumulated to date for the version.*

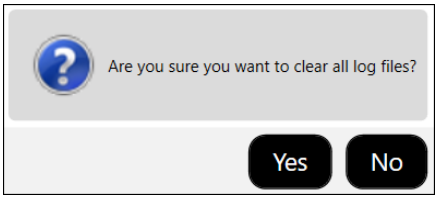
WARNING: Logs may be required to maintain a historical archive. As using the Clear All Logs button will *permanently* remove accumulated logs for the installed Matrix software version, it is recommended that you contact IT *before* clearing logs from your system.

To clear all logs:

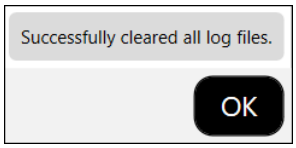
1. Tap the **Clear All Logs** button.

   Note: It is *not* recommended to clear all logs unless you are confident they are not being archived as a requirement by your organization.

2. When prompted, tap **Yes** to confirm you want to clear all logs.



3. Tap **OK** to acknowledge that all logs have been cleared.



Appendix A. Consumables

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

COUNTING CHAMBER SLIDES

Cellometer Ascend *Counting Chamber Slides* are specific to the instrument, and available in either *3-Chamber* (with loading volume of 20 µl per chamber) or *8 chamber* (with loading volume of 10 µl per chamber) formats. *8-Chamber consumables are compatible with a standard multi-channel pipette.*

Each plastic, disposable slide contains sample counting chambers with precisely controlled height. Recommended sample sizes allow for simple, automated calculation of cell concentration following imaging and counting. Image-based counting with disposable counting chambers offers several key advantages:

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

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

ASSAY REAGENTS AND KITS

Revvity provides 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 Ascend assay reagents and kits 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 Image Cytometer software, Matrix software and all related documentation. In addition, it includes a definition of *Revvity's Proprietary Information.*

WARRANTY INFORMATION

Revvity warrants that Cellometer Ascend 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 Image Cytometer software, Matrix software and any subsequent software upgrades installed on your Cellometer system by authorized representatives of Revvity, Inc. 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 Image Cytometer software, Matrix software or subsequent upgrades, and any further uses will be prosecuted by Revvity, Inc. to the maximum extent possible by law. Any other use of Cellometer Image Cytometer software, Matrix software or upgrades is explicitly prohibited. In addition, you may not disclose Cellometer Image Cytometer software, Matrix software, upgrades, or any of its features and benefits to a third party.

Revvity Proprietary Information

Cellometer Ascend 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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