USER MANUAL

CELLACA™ PLX

8003789 Rev D

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

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Cellaca™ PLX User Manual

8003789 Rev D

Updated for Matrix v6.0 Software Release
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Chapter 1. Introduction

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

CELLACA PLX SYSTEM OVERVIEW

The *Cellaca PLX Image Cytometry System* with Matrix software provides a benchtop solution for performing simple yet sensitive cell counts, viability readouts and multiplex fluorescence cell-based assay analysis in seconds. Pre-defined assays and report templates, together with dedicated reagents and consumables, enable flow-cytometry-like data analysis with no need for titrations, extra incubations, washes or decontamination.

The Cellaca PLX accurately assesses small sample volumes and displays multiple channel results with viability readouts at one minute per sample. With the Cellaca PLX, you can quickly perform cell purity and viability checks at the bench with ready-to-use immunophenotyping kits, stain transfected cell lines with viability dyes to determine percentage of live/dead transfected or transduced cells, and routinely monitor viability measurements as well as apoptosis functional assays to determine cell health. Processed samples can be moved rapidly through to downstream applications, thus preserving sample integrity.

The Matrix software used to run Cellaca PLX is pre-installed on an Operating Computer that connects to the instrument via USB cable. The system can be linked to a network for accessing external files, printers and storing 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

As primary samples used by researchers in cell and gene therapy, immunotherapy, cell line development and immuno-oncology are unique and precious due to their biological nature, the Cellaca PLX is designed to increase the efficiency of sample processing from cell staining to data readout. Intended use of the Cellaca PLX 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 *51* 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

Cellaca PLX Instrument

Instrument **Power Supply/Power Cord**

USB 3.0 Connector Cable (Figure 1)

PLX Slide Holder (Figure 2)

Cellaca PLX User Manual (PDF file on Laptop – *this document*)

Matrix Software User Manual (PDF file on Laptop)

Cellaca PLX Quick Start Guide (PDF file on Laptop and provided as printed copy with instrument)

Cellaca MX/PLX Plate Loading Template

Graphic sheet indicating mixing/loading wells for plate layouts

Cellaca MX/PLX Focus Guide

Graphic sheet to assist with adjusting instrument focus

Three **Revvity Counting Plates** (**12x2 orientation, CHM24-A100**)

Three **Revvity Counting Plates** (**3x8 orientation, CHM24-B100**)

Ten **Cellaca PLX Low Fluorescence Slides** (**CHM2-ACR**)

Two **AO/PI Viability Reagent Vials** (**CS2-0106-5mL**)

Figure 1. Connector Cable

Figure 2. PLX Slide Holder

Revvity-provided PLX Laptop (Figure 3) with **Power Supply/Power Cord**

The PLX Laptop (also referred to as the Operating Computer) ships with both the **Matrix** software and De Novo Software **FCS Express** application pre-installed. Laptop specifications are listed below.

- Windows 10
- Intel® i7 (2.1 – 4.80 GHz) Processor
- 16 Cores, 24 Threads
- 32 GB RAM
- NVIDIA RTX A2000 8GB GDDR6 Graphics Card
- 1080p Display Resolution
- 1 TB+ Hard Drive
- USB 3.0 Port

Figure 3. Laptop

De Novo Software FCS Express License Packet with Dongle

If you are setting up a new instrument, you must claim and register the FCS Express license included with the system. Follow instructions in the *De Novo Software FCS Express Flow Cytometery – 1 Dongle License 2-Year Subscription* packet included with the instrument.

MATRIX SOFTWARE OVERVIEW

The Matrix software used to run the Cellaca PLX is shared by multiple product families and includes default assays (identified with *ASD_*, *K2_*, *MX_*, *PLX_* prefixes indicating the intended instrument) and report templates for *all* supported product types. However, only assays available for the Cellaca PLX 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** workflow tab.

Key elements in Matrix software functionality are described below:

- *Acquiring Sample Data* – The **Acquire** tab launches the data acquisition workflow of two sequential screens - *Setup* and *Preview*. After loading samples, users can select a *Favorite* (with pre-defined assay parameter and consumable settings), or enter setup details, select at least one well, and click the **Preview**/**Count** button (i.e., **Count** is displayed if *Skip Preview* feature is enabled). *Preview mode allows users to preview live channel images and adjust focus/fluorescent exposure for each channel, while Count mode engages the camera to acquire sample images as specified by the assay.* The images are then processed by the Matrix imaging and pattern-recognition software to decluster, identify and count individual cells according to defined parameters.
- *Analyzing Scan Results* – The **Data** tab launches the data analysis workflow of three sequential screens – *Select*, *Results,* and *Recount*. Once a scan result is selected, users can vary the image displayed in the viewing pane by clicking available *Channel* buttons (e.g., **BR**, **FL1**, **FL2**, etc.) appearing across the top of the **Well View** tab or by choosing another well in the map. In addition, users can increase the magnification of the image and/or enable a graphic overlay to highlight counted cells. Data associated with the well appears below the image or can be viewed in a consolidated report including data from *all* wells by clicking the **Summary** tab.

  To further refine the analysis of a scan, click the **Recount** button and either select a new assay or click the **View** button to view parameters for the current assay and edit settings 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 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 Favorites, Assays, Cell Types, ACS Templates and Report Templates* – The **Manage** tab > *Favorites*, *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 list. For most entities you can also view, create or use locked entities as templates to create new ones.

Matrix Screen Elements on page *20* 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 ***Matrix Software User Manual*** for a full description of Matrix software functionality.

MINIMUM COMPUTER REQUIREMENTS

The following *minimum* requirements apply to any alternative computer (e.g., workstation or personal laptop) used to run the Matrix software in *Data Analysis* mode (i.e., while *not* connected to an instrument). *Scan results can be exported from the Operating Computer and imported onto an alternative computer for further analysis.*

- Windows 10
- Intel® i7 (2.10 – 4.80 GHz) Processor
- 16 Core, 24 Threads
- 32 GB RAM
- NVIDIA RTX A2000 8GB GDDR6 Graphics Card
- 1080p Display Resolution
- 1 TB+ Hard Drive
- USB 3.0 Port

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 in place of traditional paper-based documentation is in compliance 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 as the result of 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 ***Matrix Software User Manual*** for a full description of Matrix *21 CFR Part 11* module functionality.

ABOUT THIS USER MANUAL

This *Cellaca PLX User Manual* provides information on the following topics:

- Instrument System/Software Overviews
- Hazards, Safety and Requirements
- Components, Device SN Label and Specs
- Unpacking and Site Preparation
- Operation and Matrix Screen Elements
- Viability Methods and Assay Reagents
- Sample Preparation
- Matrix Counting and Analysis Workflow
- Cleaning, Maintenance and Storage
- Troubleshooting, Messages and FAQs
- Contacting Support and Reporting Issues
- Counting Plates/Slides, Reagents and Beads
- Report Designer for WPF Reference
- Warranty and License Details

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

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

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

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

GLOSSARY OF ABBREVIATIONS

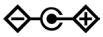
The following abbreviations may be displayed on the shipping container, on the Cellaca PLX device label or in this user manual.

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 Cellaca PLX 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**

Chapter 2. Hazards, Safety and Environment

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

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 Cellaca PLX 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. Damaged instruments or cables may result in injury to the user.

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

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

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

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

Ultraviolet Light Hazard

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

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

Servicing Hazard

No one other than Revvity-authorized personnel may service inside the protective cover of the Cellaca PLX 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 Cellaca PLX instrument should be familiar with the safety information included in this section.

Safety Protocols

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

Read all installation and operation instructions contained in this user manual thoroughly before connecting the Cellaca PLX instrument to the main power connection *prior* to use. The Cellaca PLX 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.

IMPORTANT: As the Cellaca PLX weighs approximately 44 lbs (20 kgs), two people may be required to lift and/or move the instrument if necessary.

Safety Features

The Cellaca PLX 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 Cellaca PLX 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 Cellaca PLX instrument components, device label and system specifications.

CELLACA PLX COMPONENTS

Light Bar: Display indicates when the instrument is powered on with an oscillating multi-colored light.

Stage Door: Opens to eject and closes to receive a plate (i.e., in response to users clicking the **Eject** or **Load** buttons in the Matrix software).

Power Switch: Controls power going to the instrument. *When power is on, light bar across the front is lit.*

USB 3.0 Port: Port used to connect Cellaca PLX to Operating Computer with Connector Cable provided.

Power Supply Input: Port used to connect the Power Supply/Power Cord to the instrument.

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

DEVICE SN LABEL

Polarity DC Power Connector

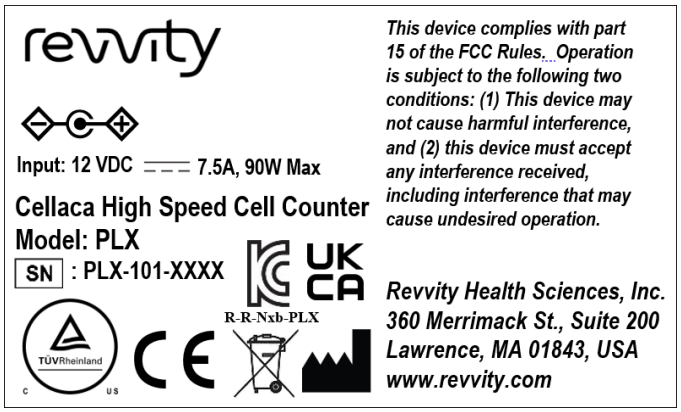
Serial Number

Korean Certification for EMC Directives and Registration Number

UK Conformity Assessment Declaration of Conformity to UK Directives

NRTL Safety Mark

European Conformity Mark



Waste Electrical and Electronic Equipment Directive (WEEE)

Manufacturer

INSTRUMENT SPECIFICATIONS

The *Cellaca PLX Image Cytometry System* is comprised of the instrument connected via USB cable to an Operating Computer used to run the instrument software. The Operating Computer can be linked to a network for accessing external files, printers and for storing count results.

	Cellaca PLX
Channels	Brightfield, Blue, Green, Orange, Red, Far Red
FL Excitation LED	370, 475, 531 and 628 nm
FL Emission Filters (Bandpass, Center Wavelength)	452, 534, 605, 655 and 692 nm
Commonly Used Compatible Dyes	7-AAD, Annexin V-FITC, AO (Acridine Orange), APC (Allophycocyanin), Calcein AM, Caspase-3 488, CFDA, DAPI, Dead Blue, Dead Green, GFP (Green Fluorescent Protein), Hoechst, KIRAVIA Blue 520*, PE (R-Phycoerythrin, PI (Propidium Iodide), RubyDead, RFP (Red Fluorescent Protein), Trypan Blue **KIRAVIA Blue 520™ is a trademark of Sony. This product is subject to proprietary rights of Sony and is made and sold under license from Sony Corporation.*
Volume (Per Well)	15 µL (slide volume) 50 µL - 200 µL (plate volume)
Cell Size/ Diameter Range	5 - 80 µm
Concentration Range	$1x10^5$ - $1x10^7$
Automation Ready?	Compatible with robotic integration and automated liquid handling systems
Dimensions (H x W x D)	13 in x 13 in x 16 in (33 cm x 33 cm x 40.6 cm)
Weight	44 lbs (20 kgs)
Input to Power Adapter	100-240 VAC, 50/60 Hz, 1.5 A
Output to Instrument	12 VDC, 7.5 A

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

DECLARATION OF CONFORMITY

The Cellaca PLX conforms to appropriate country standards and governing regulations as listed in the *Declaration of Conformity* provided by Revvity, Inc. (manufacturer of Cellaca instruments). To request the Cellaca PLX *Declaration of Conformity*, contact Support by visiting https://www.revvity.com/contact-us or send an email to: CellC-support@revvity.com

Chapter 4. Unpacking and Site Preparation

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

UNPACKING/SETTING UP THE INSTRUMENT

Congratulations on your purchase of one of the fastest high-throughput cell counters available! Please ensure you have secured the space required to set up your new Cellaca PLX (see *Facility Requirements* on page *17*).

For assistance in setting up the instrument, visit the *Cellaca MX* page on our website for a training video on unboxing and getting started. *Steps for unboxing and getting started with Cellaca MX/PLX instruments are the same, and both instruments are powered by the Matrix software.*

Our staff would be happy to help you set up your Cellaca PLX. 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 65 lbs (30 kgs).*
3. Open the outer box and remove protective packaging.
4. Remove the Operating Computer (laptop) box and set aside.
5. Remove the Consumables box and carefully open to locate any temperature-controlled beads/reagents. Store the beads/reagents per the product insert provided.
6. Remove instrument accessories (e.g., Power Supply/Cord, USB 3.0 Connector Cable, PLX Slide Holder, *Cellaca MX/PLX Plate Loading Template*, *Cellaca MX/PLX Focus Guide*, printed version of *Cellaca PLX Quick Start Guide* as well as any other documents) and set aside.
7. Remove the Cellaca PLX instrument and place it in the prepared area (see *Facility Requirements* on page *17*).
8. Remove the protective plastic from around the Cellaca PLX.
9. Connect the instrument Power Supply to the instrument Power Cord.
10. Connect the instrument Power Supply to the Cellaca PLX and then plug instrument Power Cord into a surge protector (recommended) or dedicated electrical outlet.
11. Turn the Cellaca PLX Power Switch to *ON* position. Power Switch is located on back of instrument (on lower right side when facing the front). *Light bar across front will be lit.*
12. Open the Operating Computer box and set up laptop within reach of the Cellaca PLX. *The USB 3.0 Connector Cable must reach laptop while it is connected to instrument.*
13. Connect the laptop Power Supply to the laptop Power Cord.
14. Connect the laptop Power Supply to the Operating Computer and then plug the laptop Power Cord into an electrical outlet.
15. Power on the Operating Computer and wait for it to initialize.

16. Connect the Operating Computer to the Cellaca PLX using the USB 3.0 Connector Cable.

    Note: When connecting the computer to the instrument, users must wait until the instrument makes an audible click (i.e., indicating the instrument motors are communicating with the computer) *before* launching the software. Not waiting for this click can result in errors during the startup sequence. *Keep in mind this note will apply each time the computer and instrument are disconnected/connected, or powered off/on again.*

17. If prompted by the software, log in to the Operating Computer.
18. Launch the Matrix software from the desktop of the Operating Computer. The system is now ready for use.

Cellaca PLX IQ/OQ Validation

Revvity has designed an *Installation Qualification/Operation Qualification (IQ/OQ)* validation specifically for the Cellaca PLX. 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

Matrix 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 *Cellaca PLX IQ/OQ*.

Note: Integrating the Matrix *21 CFR Part 11 OQ Protocol Supplement* into the *Cellaca PLX IQ/OQ Protocol* requires that you have purchased a Matrix 21 CFR Part 11 module license and the *Cellaca-21PLX-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

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 Cellaca PLX.

Due to the nature (biological liquid) and positioning of sample, the instrument should be placed on a level surface.

Ensure instrument has room to eject the stage and allow for proper airflow around the instrument to prevent overheating. Keep the area around the Cellaca PLX clean between, during and post operation.

Electrical requirements for the Cellaca PLX are as follows:

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

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

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

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

Transporting the Instrument

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

Due to the size and weight of the Cellaca PLX, special care should be taken when moving the instrument. When instrument is loaded into a 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

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Chapter 5. Operation

This chapter describes first-time use of the Cellaca PLX instrument and presents an overview of Matrix software screen elements. For more information on performing a count and analyzing scan results, see *Chapter 8. Matrix Counting and Analysis Workflow* on page *31*.

Note: Confirm that the following conditions have been met prior to launching the Matrix software:

- Prior to launching the Matrix software, be sure to verify your system is set up as recommended in *Chapter 4. Unpacking and Site Preparation* starting on page *15*. In addition, follow all equipment safety protocols while using the instrument and keep the area around it clean before, during and after operation.
- When connecting the computer to the instrument, users must wait until the instrument makes an audible click (i.e., indicating the instrument motors are communicating with the computer) *before* launching the software. Not waiting for this click can result in errors during the startup sequence. *Keep in mind this note will apply each time the computer and instrument are disconnected/connected, or powered off/on again.*

USING INSTRUMENT FOR THE FIRST TIME

Launching the Matrix Software

1. From desktop of Operating Computer, launch the Matrix software by double-clicking the **Matrix** icon. *The instrument will run through a startup sequence that includes connecting to the database and initializing the calibrations*.

2. Click the **Home** tab followed by the **About Matrix** button to display software version and Support contact information.

   Note: If the Matrix *21 CFR Part 11* module has been enabled for your system, users must log in *before* they can use the software.

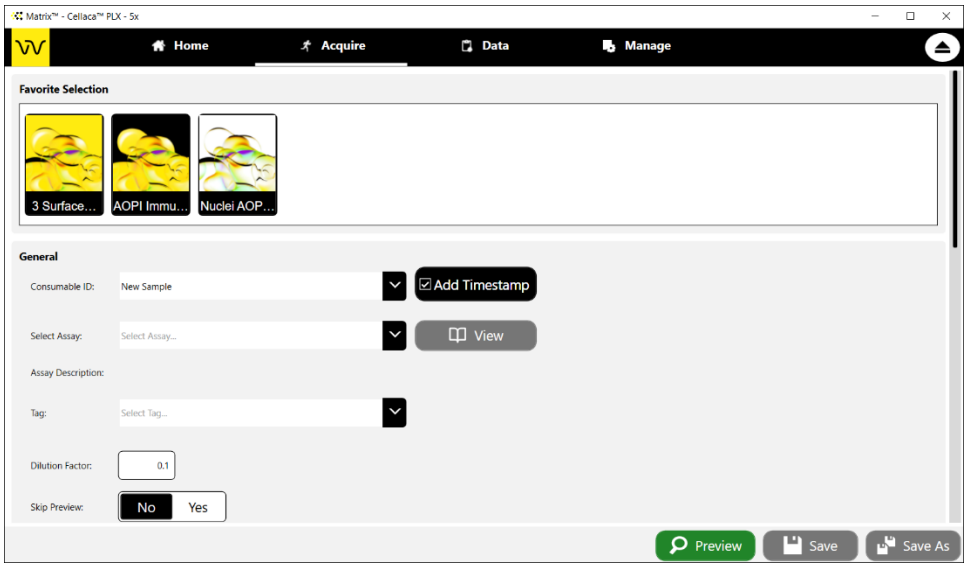
Calibrating Instrument via the Software

After initial setup of an instrument, it must be calibrated using the Matrix software prior to first-time use. *You will be prompted with a message if you attempt to acquire data before calibration has been performed.*

The calibration process takes a background image that will be used to normalize the cell counter for each installed filter pair *without* a consumable counting plate or slide holder loaded in the instrument. For assistance, contact Support by visiting https://www.revvity.com/contact-us or send an email to: CellC-support@revvity.com

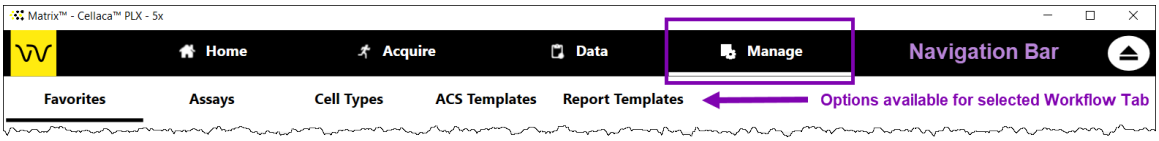
MATRIX SCREEN ELEMENTS

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 clicking the* ***Acquire*** *workflow tab.* Basic screen elements are described below.



Navigation Bar

The *Navigation Bar* visible across the top of the screen is always displayed.

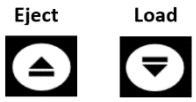


Functionality of Navigation Bar elements is described below.

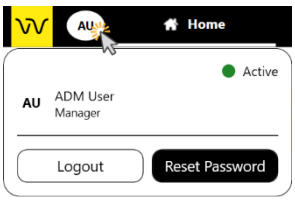
Workflow Tabs: The **Home**, **Acquire**, **Data** and **Manage** tabs represent key areas of functionality as described in *Workflow Tabs* on page *21*. Clicking a tab may display options for that tab (if available) in a sub navigation bar.

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

Eject/Load Buttons: Control movement of the instrument stage (either ejects stage from instrument or retracts stage into instrument for sample loading) so the consumable can be placed onto or removed from the stage. ***Eject*** *button becomes temporarily disabled while the instrument performs the count process.*



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. Clicking the User ID displays the User ID Card which contains the **Logout** and **Reset Password** buttons. See the ***Matrix Software User Manual*** for details on using module functionality.



Workflow Tabs

Functionality associated with each workflow tab is described below. Note that clicking 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 logo for the instrument to which you are connected and the Matrix software logo. 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 *56*.

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 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.

Use the **Back** button 

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

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 *38* 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. *Clicking 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 allow users to run previously saved counting parameter settings to instantly run samples without having to choose an application, thus bypassing the setup process and the Preview screen (if *Skip Preview* feature is enabled).

- 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 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 ***Matrix Software User Manual*** for details on using **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 allow users to run previously saved counting parameter settings to 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 click 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.

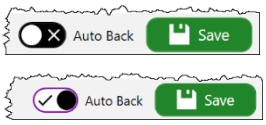
STREAMLINED USER EXPERIENCE

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

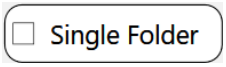
Add Timestamp Button: When entering setup details, clicking the **Add Timestamp** button automatically appends a timestamp to the Consumable ID when the scan result is created. *This button is enabled by default.*

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 clicked. Click 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. *Clicking 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. *Clicking 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 focusing methods.

MANUAL FOCUS

The traditional *Manual* focusing operation allows users to choose the initial *Z* position and then make small adjustments using focusing controls (i.e., **Coarse Focus Manual Offset** and **Fine Focus Manual Offset** buttons) in the Preview Screen. This method used a *Focus Map* option to change the *Z* plane based on the consumable itself.

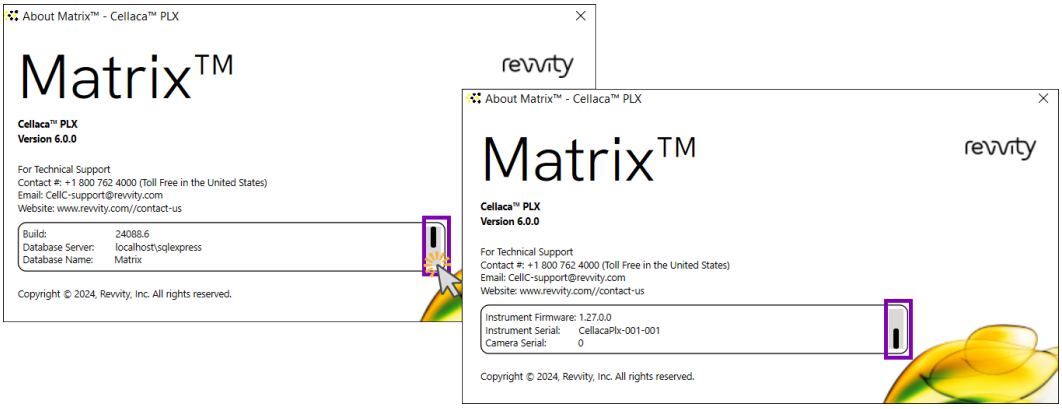
IMAGE-BASED AUTO FOCUS

The image-based auto focusing operation depends on the contrast within an image. When a consumable is scanned, the software analyzes objects in the image, selects images with the best contrast, and then applies an algorithm to sharpen the focus further. Users have the option to perform image-based auto focusing for each of the brightfield and fluorescent channels.

VIEWING SOFTWARE VERSION

The **Home** tab displays the logo for the instrument to which you are connected. As the Cellaca PLX instrument is run using the Matrix software, the Powered By Matrix™ logo is displayed.

The **Home** tab also contains the **About Matrix** button which displays 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/camera serial numbers). *Users will need to click the scroll bar to view all information.*



For details on using the **Generate Diagnostic Report** and **Clear All Logs** buttons to assist the Support team with troubleshooting technical issues, see *Generating Diagnostic Reports* on page *56*.

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 and Assay Reagents

This chapter describes how to choose a viability staining method based on the cell sample and selected assay type. In addition, it provides an overview of available ready-to-use assay PLX assay reagents and kits.

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 *prior* to staining.

Note: Cell concentrations of 1.0 x 10^5 – 1.0 x 10^7 cells/mL can be analyzed on the Cellaca PLX, with a concentration of 1.0 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 Method* 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 50 µL of cell sample with 50 µL of a 0.2% trypan blue staining solution (for a final concentration of 0.1% trypan blue). Gently mix by pipetting up and down 10x.

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

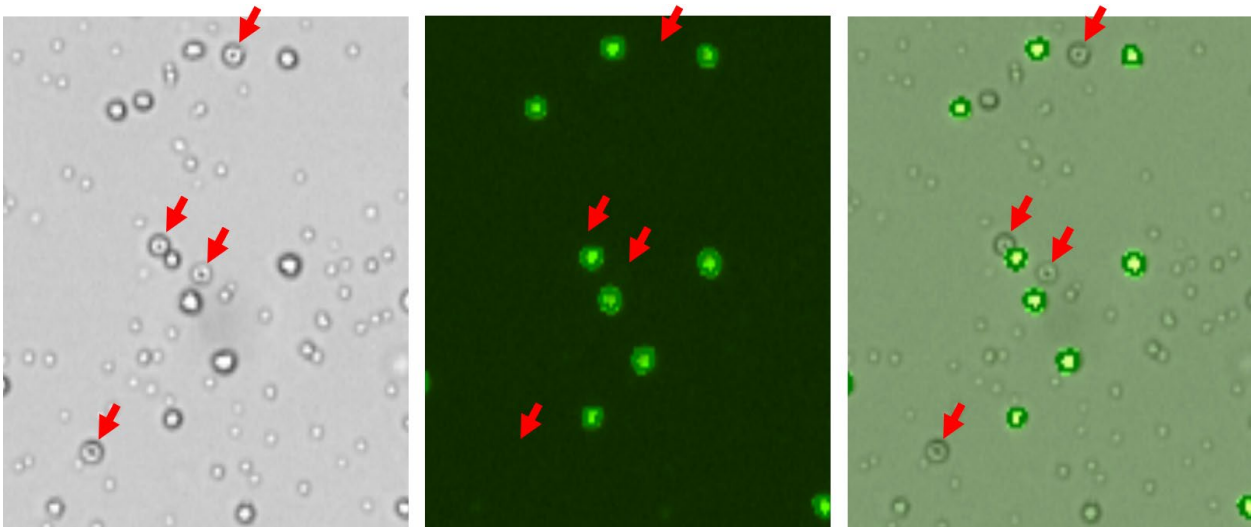
Using AO/PI Viability Method

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 Cellaca PLX 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 50 µL of cell sample with 50 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 plate loading wells.

The table below shows recommended dilutions when preparing cell samples for AO/PI viability analysis and the final *Dilution Factor* to enter into the Matrix software for a few 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	50 µL	50 µL	20
PBMCs following Ficoll separation	*Not Required*	50 µL	50 µL	2
Mononuclear cells from processed bone marrow	*Not Required*	50 µL	50 µL	2
Tumor digest/Tissue digest	*Not Required*	50 µL	50 µL	2
Stem cells from CD34+ separation	*Not Required*	50 µL	50 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

PLX ASSAY REAGENTS AND KITS

Revvity offers a variety of ready-to-use assay reagents and reagent kits to accurately perform fluorescence-based assays for surface marker detection, apoptosis, cell counting and viability. Available kits include:

- Single Surface Marker
- Single Isotype Control
- Single Surface Marker Viability
- Two Surface Marker Total Cell
- Two Surface Marker Dead Cell
- Two Surface Marker Viability
- Three Surface Marker Total Cell
- Three Surface Marker Dead Cell
- Apoptosis
- Viability for Fluorescent Protein-expressing 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 Cellaca PLX assay reagents and kits directly from Revvity.

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Chapter 7. Sample Preparation

This chapter describes preparing counting plates and/or low fluorescence slides with counting beads or cell samples.

PREPARING PLATES/SLIDES WITH SAMPLES

To prepare plates/slides with counting beads or cell samples:

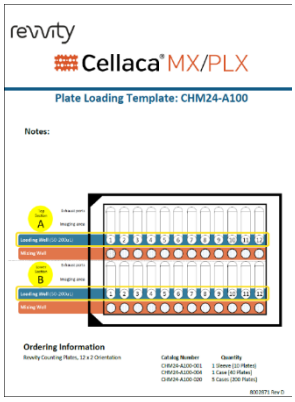
1. On the desktop of Operating Computer, double-click the **Matrix** icon to launch the software.

The instrument will run through a startup sequence that includes connecting to the database and initializing the calibrations.
2. Obtain a Revvity counting plate (*in either 12x2 or 3x8 layout)* or the PLX slide holder with up to four (4) low fluorescence slides.
3. Invert counting bead solution or tube containing cell sample a total of 10 times (10x). *If using counting beads, vortex bead solution for 10 seconds. Do not vortex cell samples.*

Note: Cell concentrations of 1.0 x 10^5 – 1.0 x 10^7 cells/mL can be analyzed on the Cellaca PLX, with a concentration of 1.0 x 10^6 cells/mL being optimal.

TO LOAD A COUNTING PLATE

1. Place the *Cellaca MX/PLX Plate Loading Template* on the lab bench with the appropriate layout facing up and set a counting plate on top of the template graphic, aligning the notched corner of the plate. *You should be able to view and identify the mixing/loading wells through the plate.*



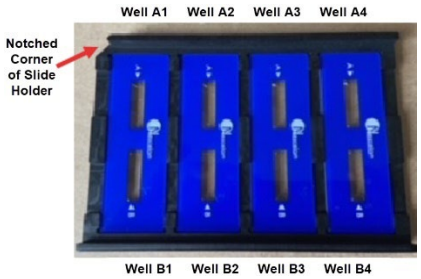
   Note: Read the Revvity consumable plate product insert to better understand the Well Map and wells to use when pipetting samples.
2. Set pipette to 50 µL and then pipette bead solution/cell sample up and down 10x to break up any potential clumps. Load 50 µL of bead solution/cell sample into each of the individual *Loading Wells* on the counting plate. *Liquid will move into imaging area for each filled well.*

   If cell samples require mixing prior to loading, pipette samples into *Mixing Wells* on the counting plate (adjusting volume as necessary to account for dilution factor). Once mixing of samples is complete, pipette 50 µL from *Mixing Wells* into associated *Loading Wells*.

TO LOAD SLIDES AND INSERT INTO SLIDE HOLDER

1. Place up to four PLX low fluorescence slides on unused/clean Kimwipes.
2. Since two samples can be loaded per slide, it is recommended that you label individual chambers in the blue area using a marker. Take care to ensure that clear optical windows of wells are *not* touched.

3. Set Pipette to 15 µL and then pipette bead solution/cell sample up and down 10x to break up any potential clumps. Load 15 µL of sample into induction port of a slide well. *Liquid will move into imaging area for each filled well.*
4. Insert up to four prepared slides into slide holder, aligning well *A* of each slide with the top of holder (indicated by notched corner).



PLACING A PLATE/SLIDE HOLDER ONTO INSTRUMENT STAGE

1. In the Matrix software, click the **Eject** button located in the Navigation Bar to eject stage from instrument. Place a prepared counting plate onto extended stage, taking care to align notched corner of plate with top left corner of stage (i.e., well *A1* is positioned in top left corner while facing the stage).

When using a slide holder, place holder containing up to four low fluorescence slides onto extended stage, taking care to align notched corner of holder with top left corner of stage (i.e., well *A1* is positioned in top left corner).

2. Click the **Load** button now available in the Navigation Bar to retract stage into instrument.

Chapter 8. Matrix Counting and Analysis Workflow

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

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

PERFORMING A COUNT

Performing a count consists of multiple steps (e.g., *Loading Samples*, *Choosing Data Acquisition Workflow – Selecting a Favorite* or *Entering Parameter Settings* – and *Previewing Samples*) before clicking the **Count** button.

Loading Samples

If you have already loaded a consumable counting plate or slide holder with samples using instructions from Preparing Plates/Slides with Samples *on page 29, skip to Choosing Data Acquisition Workflow, below.*

1. Power on the Operating Computer and Cellaca PLX instrument.

   Note: When connecting the computer to the instrument, users must wait until the instrument makes an audible click (i.e., indicating the instrument motors are communicating with the computer) *before* launching the software. Not waiting for this click can result in errors during the startup sequence. *Keep in mind this note will apply each time the computer and instrument are disconnected/connected or powered off/on again.*

2. From the desktop of Operating Computer, double-click the **Matrix** icon to launch the software. *The instrument will run through a startup sequence that includes connecting to the database and initializing the calibrations.*

3. In the Matrix software, click the **Eject** button located in the Navigation Bar to eject stage from instrument. Place a prepared plate or slide holder containing slides onto extended stage, taking care to align notched corner of plate/slide holder with top left corner of stage (i.e., well *A1* is positioned in top left corner).

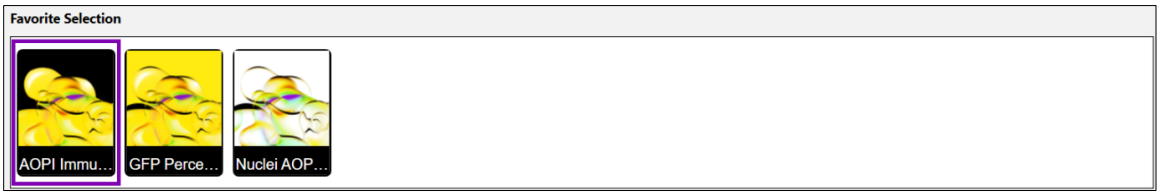
4. Click the **Load** button now available in the Navigation Bar to retract stage into instrument.

Choosing Data Acquisition Workflow

1. In the Navigation Bar, click the **Acquire** tab (if not already displayed by default) to display the Setup screen.
2. Either select a favorite from the **Favorite Selection** panel or enter parameter settings for a count in the *General*, *Consumable Details*, *Well Names*, and *Reports and Exports* areas.

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 click the **Preview** button (if enabled; skip to *Previewing Samples* on page *35*) or the **Count** button (see *Clicking the Count Button* on page *37*) to proceed.

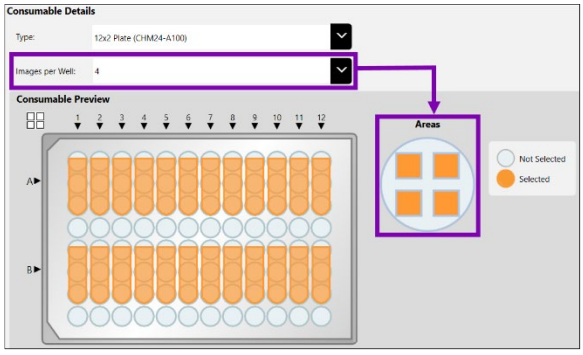
To run an assay available in the dropdown, users must enter parameter settings before performing a count.

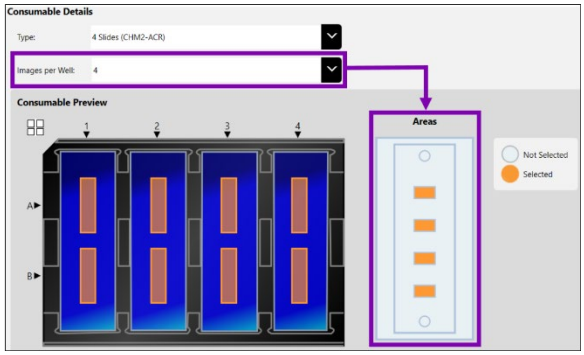
Entering Parameter Settings

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. Select an assay from the drop-down and confirm the assay description displayed.

   To view and/or edit assay details, click the **View** button. Click **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 click **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 to performing the count.
5. In the *Consumable Details* area, choose consumable **Type** and number of **Images Per Well**.
6. In the *Consumable Preview* area you are required to select the loaded wells in the Well Map. To select individual wells, click to select or de-select accordingly. To select a block of wells, click on a well at the beginning of block and hold button down while dragging mouse to the end of block before releasing button. To select or de-select *all* wells, click the **All Wells** button.





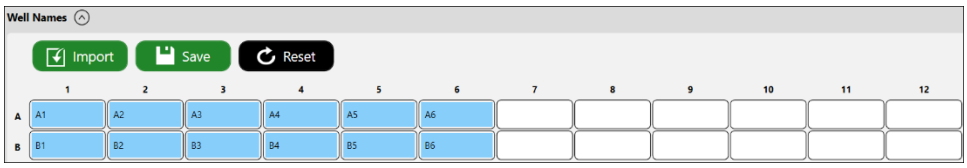
The *Consumable Details* area allows you to choose consumable **Type** (e.g., *4 Slides* contained in slide holder, *8x3 Plate,* or *12x2 Plate*) and specify the number of **Images Per Well** (i.e., *1* or *4*) to be captured during the scan. An *Images Per Well* indicator appears in the lower right corner of the Well Map and depicts the selected number of images to be captured for each well.

Note: If *4 Images Per Well* is selected, users will be able to click on four separate areas (i.e., representing quadrants) of a well in both the Preview and Results screens. This option allows users to take four independent images of a well to be used for viewing and export, rather than one large image of the whole well, thus 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 quadrant filename indicating its position index for the area in the slide chamber/well.

In the *Consumable Preview* area you are required to select the loaded wells in the Well Map. To select individual wells, click to select or de-select accordingly. To select a block of wells, click on a well at the beginning of block and hold button down while dragging mouse to the end of block before releasing button. To select or de-select *all* wells, click the **All Wells** ☐☐ button.

NAMING WELLS

Once well selection is complete, you can expand the *Well Names* area below the map to see the corresponding well name fields also highlighted in blue. These fields allow you to enter well names or import them from a file to identify samples loaded into each well.



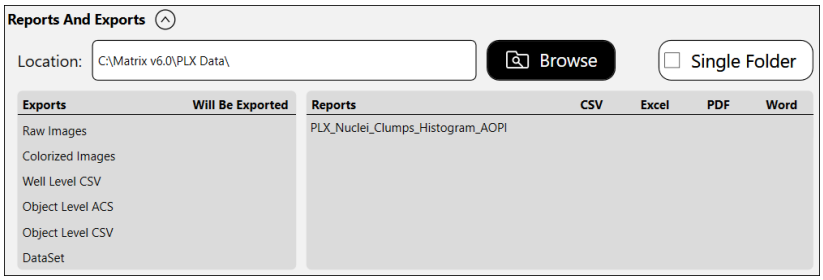
To manually enter well names, simply click on each highlighted field individually and type in a name for that well. As an alternative, you can use the following buttons to import, save and reset selected well names.

Import	Allows users to import a previously saved *.csv* file containing well names. Click the **Import** button, navigate to where *.csv* file is stored, select the file and click **Open** to load well names.
Save	Allows users to save the currently displayed well names as a file that can be loaded back into the system for use with another plate. Click the **Save** button and navigate to where the *.csv* is to be stored. Enter a name for the file and click **Save**, followed by clicking **OK** in response to the confirmation prompt. *Users can open the file in a .csv compatible application (such as MS Excel) and rename the wells before importing the file again.*
Reset	Allows users to clear the currently displayed well names. Click the **Reset** button followed by clicking **Yes** in response to the confirmation prompt. *Well names that have been cleared will remain highlighted in blue for your reference.*

Note: If you are creating a time course series of data for use in custom reporting, it is critical that well names be consistent for all scan results in the series. See the ***Matrix Software User Manual*** for custom reporting details.

SETTING AUTO EXPORT LOCATION

Expand the *Reports and Exports* area to specify a **Location** for automatic exports of images/data and generated output files for reports. Click 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* (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, click the **View** button and expand the *Reports and Exports* option. Select *Exports* by clicking 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 ***Matrix Software User Manual*** for details on editing an assay to manage exporting and reporting options.

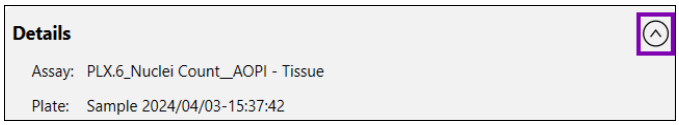
Previewing Samples

Once you have completed entering setup details for the samples, click the **Preview** button (if enabled) located at the bottom of the Setup screen. 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 fluorescence exposure for each channel.

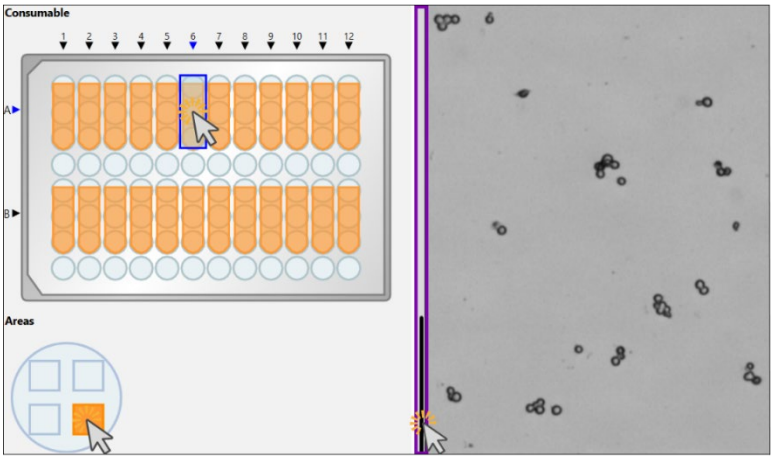
Note: If the **Skip Preview** field in the Setup screen is enabled (i.e., set to **Yes**), the software skips previewing samples and displays the **Count** button instead. See *Clicking the Count Button* on page *37* for details.

PREVIEWING LIVE IMAGES

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



In the *Consumable* area, click on highlighted wells to view live images of samples contained in the plate or PLX low fluorescence slides.



If an **Images Per Well** value other than *1* was selected, you can also select a specific quadrant within a well by clicking on the indicator below the Well Map. *Use the scroll bar to view this indicator.* As you move from well to well (and from quadrant to quadrant), the live image changes per selected well and quadrant.

To zoom in/out of an image, move mouse to hover cursor over the viewing pane and turn the scroll wheel. To move a zoomed image around, click and drag the image to a new location as needed.

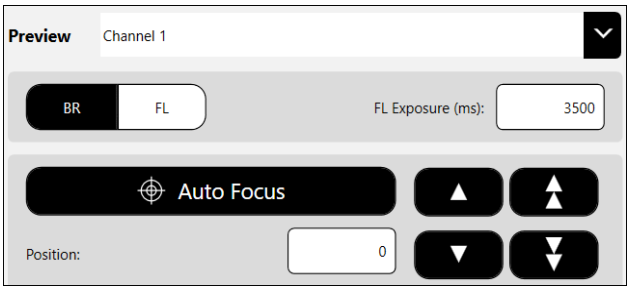
ADJUSTING FOCUS

To adjust the focus of a live image being previewed, use the *Focus* controls as indicated below. Obtaining good focus is key to ensuring accurate cell counts.

In the **Preview** field, channels available for viewing (e.g., *Channel 1, Channel 2*, etc.) are based on the assay Imaging Mode. The *Channel 1*/*BR* image is displayed by default. Clicking the **FL** button displays the *Channel 1*/*FL* image. To view images for another channel, select it from the **Preview** field drop-down. Click the **FL** button to view fluorescence in that channel.

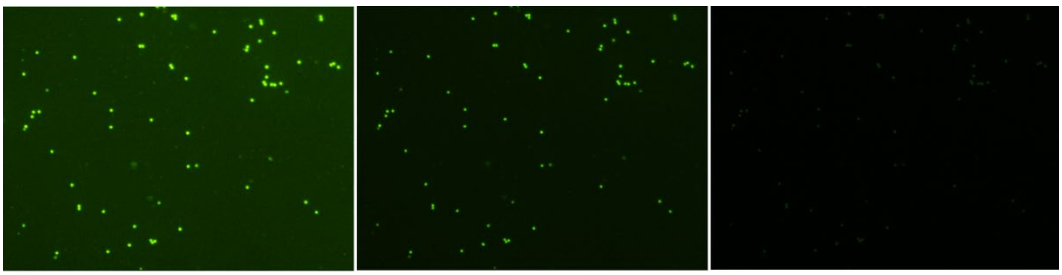
Notes:

- If only one channel is available, the *Preview* field channel drop-down will be hidden. In addition, if the *BR Only* Imaging Mode is selected, the **FL** button will also be hidden.
- 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*, *BR2*/*FL2*, etc.
- When working with assays that have more than one channel, use the *Channel 1*/*BR* image to adjust focus and then select the *FL* image to confirm exposure. For *Channels 2-6*, you only need to select the *FL* images to confirm exposure since focus of their paired *BR* images is adjusted automatically when you performed the task for *Channel 1*.



Auto Focus	Allows the instrument to determine the best focal position for the selected well.
Position	Allows users to enter a numerical value for the vertical (Z) position of the objective lens.
Fine Focus Manual Offset Controls	Allows users to finely adjust the vertical (Z) position of the objective lens for optimal focus (in µm). Click the up/down arrow to adjust focus accordingly. 
Coarse Focus Manual Offset Controls	Allows users to coarsely adjust the vertical (Z) position of the objective lens for optimal focus (in µm). Click the up/down double-arrow to adjust focus accordingly. 

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.



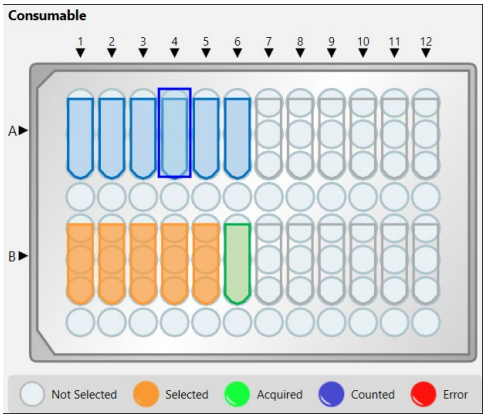
Exposure Too High | Good Exposure | Exposure Too Low

Clicking the Count Button

Once you have completed previewing the live image for the sample, click 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.

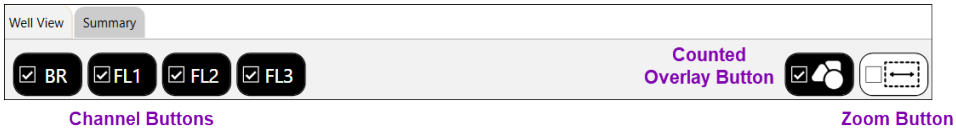
Note: Depending on the number of selected wells/images per well and defined assay parameters, the counting process can take a few seconds to up to a few minutes.

As the system acquires sample images and calculates count results, the colors used to mark selected wells/images per well will change to indicate status (i.e., from *Selected* to *Acquired* to *Counted*) as shown in the legend appearing below the Well Map.



You can click on a well as soon as it is *Counted* to display results below the viewing pane. *Count results will be displayed, printed and exported based on 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.

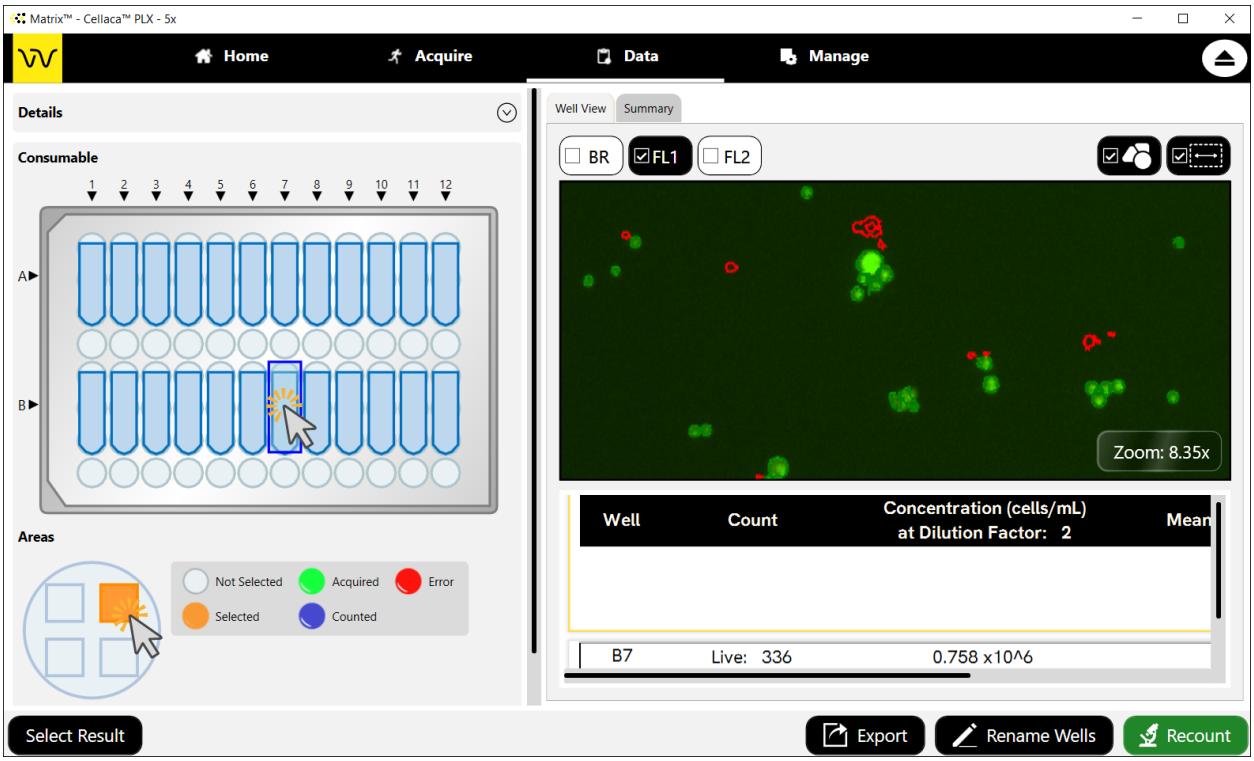


To zoom in and out of sample images, move the mouse to hover cursor over the viewing pane and turn the scroll wheel. Current *Zoom* magnification is displayed in bottom right corner of viewing pane.

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

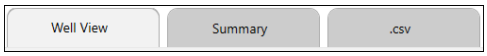
When analyzing scan results, a single image is displayed in the viewing pane for the sample in the selected well. To display other acquired images, click on highlighted wells in the Well Map.



If *4 Images Per Well* was selected, users can click on four separate areas (i.e., representing quadrants) of a well. This option allows users to take four independent images of a well to be used for viewing and export, rather than one large image of the whole well, thus 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 quadrant filename indicating its position index for the area in the slide 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 ***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. *If multiple images were taken (based on your Images Per Well selection), they will each represent a separate area in the well (e.g., 4 images per well will result in an image of each quadrant).* Click and drag in the image to view other locations in the well. To display images for other acquired samples, select other highlighted wells in the Well Map.

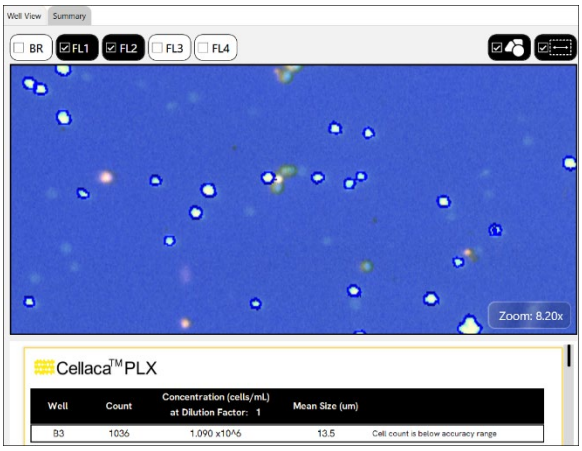
To zoom in and out of the image, move mouse to hove cursor over the viewing pane and turn the scroll wheel. If **Zoom** button is enabled, *Zoom* magnification is displayed in bottom right corner of viewing pane and can be increased up to 10.00x.

At the bottom of the **Well View** tab is a report containing well-level details for the sample in the selected well.

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.

- Click the *Brightfield* (**BR**) or *Fluorescence* (e.g., **FL1**, **FL2**, **FL3**, **FL4** etc.) buttons to select/de-select channels used in the image display. *Channel views are overlaid on top of each other.*
- Click 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). For Expression assays, Blue is used for outlining total cells in the masked channel.*
- Click the **Zoom** button to enable/disable display of the current *Zoom* magnification in bottom right corner of viewing pane. *Zoom feature will still be functional even if not displayed.*

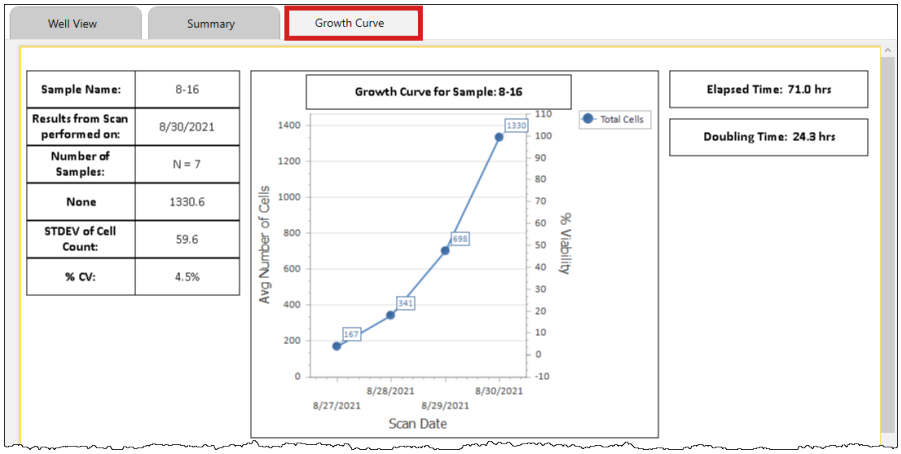


Understanding Custom Reporting

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 new scan or recount is performed.

For example, a custom **Growth Curve** report tab has been enabled in the scan result shown below. Clicking 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).

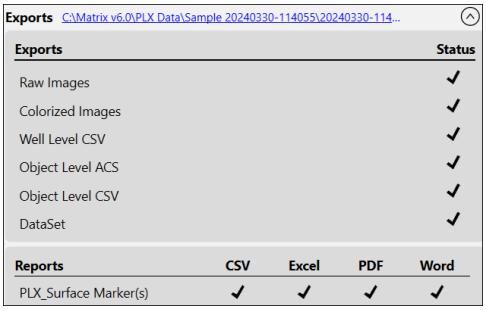


See the ***Matrix Software User Manual*** for custom reporting details.

EXPORTING/PRINTING SCAN RESULT DATA

Verifying Auto Exports

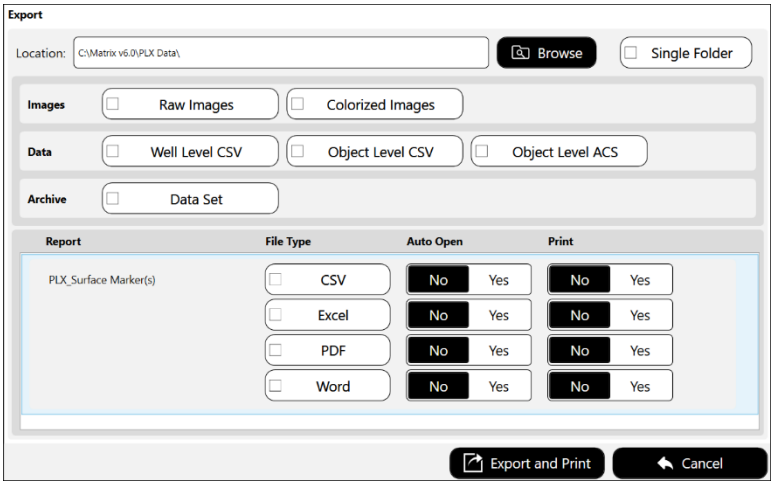
Expand the *Exports* area in the Results screen to verify that automatic exports were completed and click the location link to open folder where the exported scan result files are stored.



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, click the **Export** button located at the bottom of the Results screen. The Export dialog is displayed.

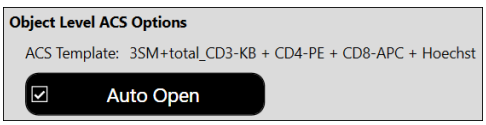


Confirm the export **Location** and if necessary update this path by clicking the **Browse** button, navigating to a folder on your Operating Computer or network, and clicking **OK**.

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.

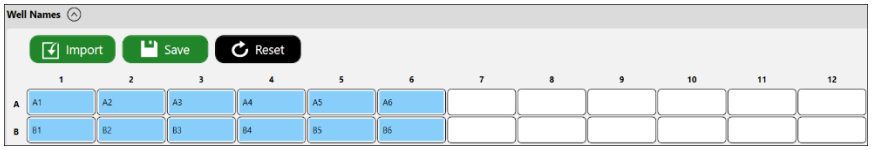
Note: Any changes to export options are applied only when manually exporting scan results (i.e., by clicking the **Export and Print** button) and will *not* be saved with the assay or scan result.

RENAMING WELLS

You can rename wells as necessary for a scan by clicking the **Rename Wells** button located at the bottom of the Results screen. Wells that were selected for the scan are highlighted in blue and contain the current well names. *All remaining wells will be blank and disabled for editing.*

Note: As well names are associated with a scan and *not* individual results derived from that scan, renaming wells in a scan result will affect well names used for *ALL* results (counted or recounted) generated from that scan.

To manually enter well names, simply click on each highlighted field individually and enter a name for that well.



As an alternative, you can use the following buttons to import, save and reset selected well names.

Import — Allows users to import a previously saved *.csv* file containing well names. Click the **Import** button, navigate to where *.csv* file is stored, select the file and click **Open** to load well names.

Note: Using this button when editing a scan result imports only those well names that were selected/enabled for the scan. *All other well names in the imported file will be ignored.*

Save — Allows users to save the currently displayed well names as a file that can be loaded back into the system for use with another plate. Click **Save** and navigate to where the *.csv* is to be stored. Enter a name for the file and click **Save**, followed by **OK** in response to confirmation prompt. *Users can open the file in a .csv compatible application (such as MS Excel) and rename the wells before importing the file again.*

Reset — Allows users to clear the currently displayed well names. Click **Reset** followed by **Yes** in response to the confirmation prompt. *Well names that have been cleared will remain highlighted in blue for your reference.*

After any changes to well names have been saved, reports enabled as tabs in the Results screen will immediately be updated to display the new well names.

Note: If you are creating a time course series of scan cell counts, it is critical that well names be consistent for *all* scan results in the series. See the ***Matrix Software User Manual*** for custom reporting details.

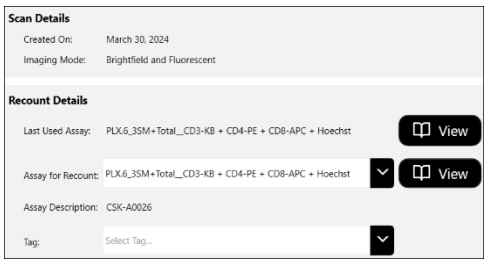
PERFORMING A RECOUNT

If you find it necessary to fine-tune assay parameters after reviewing your data results, click 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), click 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

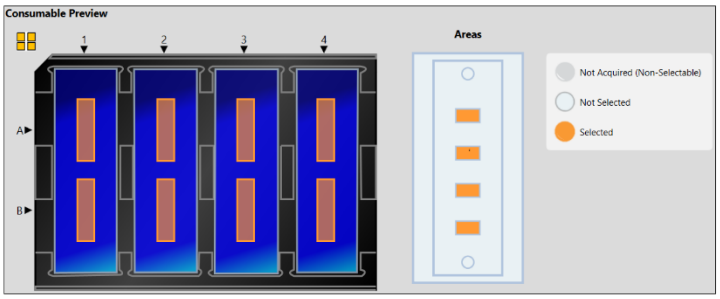
In the *Recount Details* area you can view parameter settings for the *Last Used* assay, select a new assay from the drop-down or view the current assay to edit parameter settings to be used for the recount.



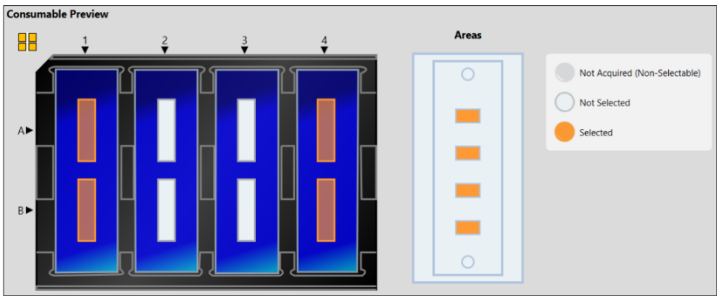
De-selecting Wells

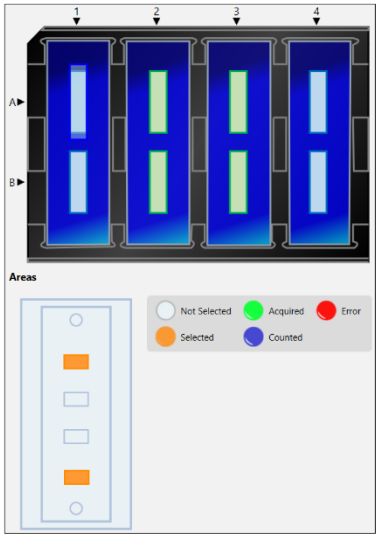
In the *Consumable Preview* area you can de-select wells previously highlighted to exclude them from the recount (e.g., to increase counting speed). *You cannot add wells to the recount that were not selected for the initial count.*

Wells selected for initial Count



Wells de-selected for Recount





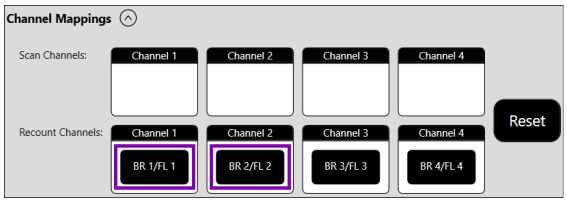
Well status updated after Recount

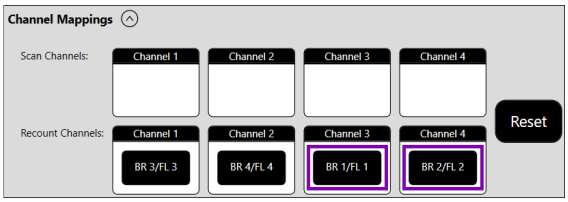
Managing Channel Mappings

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: With the exception of *Brightfield Only* configurations (i.e., *BR1*, *BR2*, etc.), each channel will have separate brightfield and fluorescent images as depicted in the mapping indicators (i.e., *BR1/FL1*, *BR2/FL2*, etc.).

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

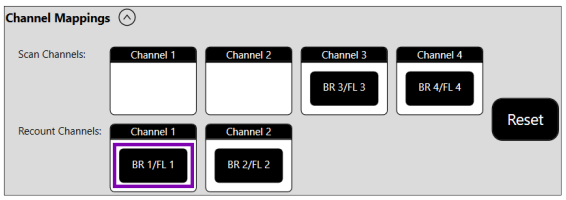


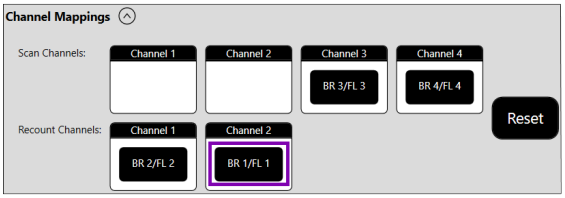


To return channel mappings to the positions they were in when you first expanded the *Channel Mappings* area, click the **Reset** button followed by **Yes** to confirm the action.

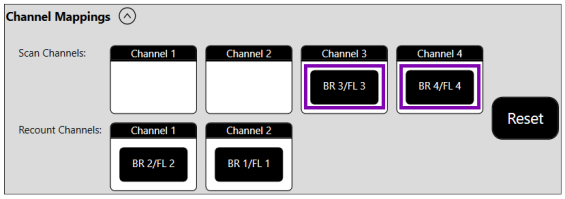
If a new assay selected for recount has fewer channels or you edit the assay previously used to reduce the number of channels, mappings displayed are updated to reflect available channels. To manage channel mappings for use in the recount, click on mapping indicators and drag them to an available position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

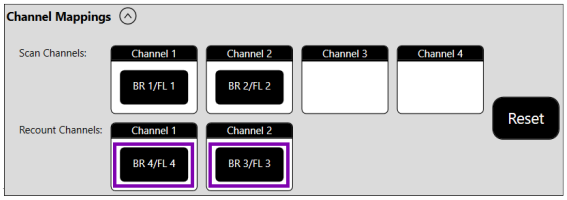
4 Channel Assay Reduced to 2 Channel for Recount



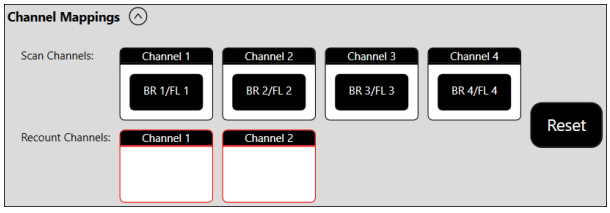


If dragging an indicator from the *Scan Channels* to *Recount Channels* area and the *Recount Channels* postion is already populated, the indicator in *Recount Channels* will be returned to its home *Scan Channels* location.



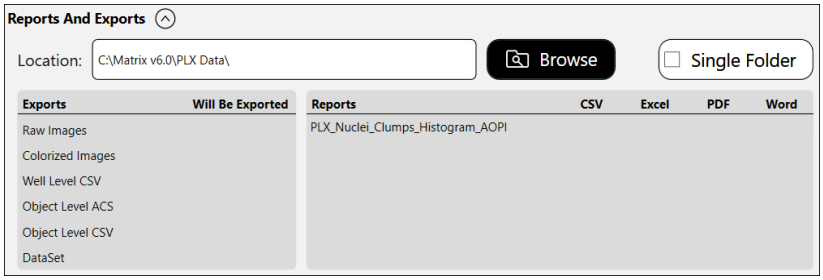


If you drag indicators from *Recount Channels* to their home *Scan Channels* locations, a red outline is used to highlight empty *Recount Channels* locations. All *Recount Channels* must contain an indicator prior to recount.



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. Click 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 checkmark.

Note: To modify *Report and Export* settings displayed, click 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.

Clicking the Recount Button

Once you have completed entering recount details for the samples, click 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

- When entering *Well Details* in the Setup screen, you must select wells in the consumable containing your samples before clicking the **Preview** button. To select wells individually, click on each well to select or de-select it accordingly. To select a group of wells, click on a well at the beginning of the group and hold the mouse button down while dragging your mouse to the end of the group before releasing it. To select or de-select all wells, click the **All Wells** ⊞ button.

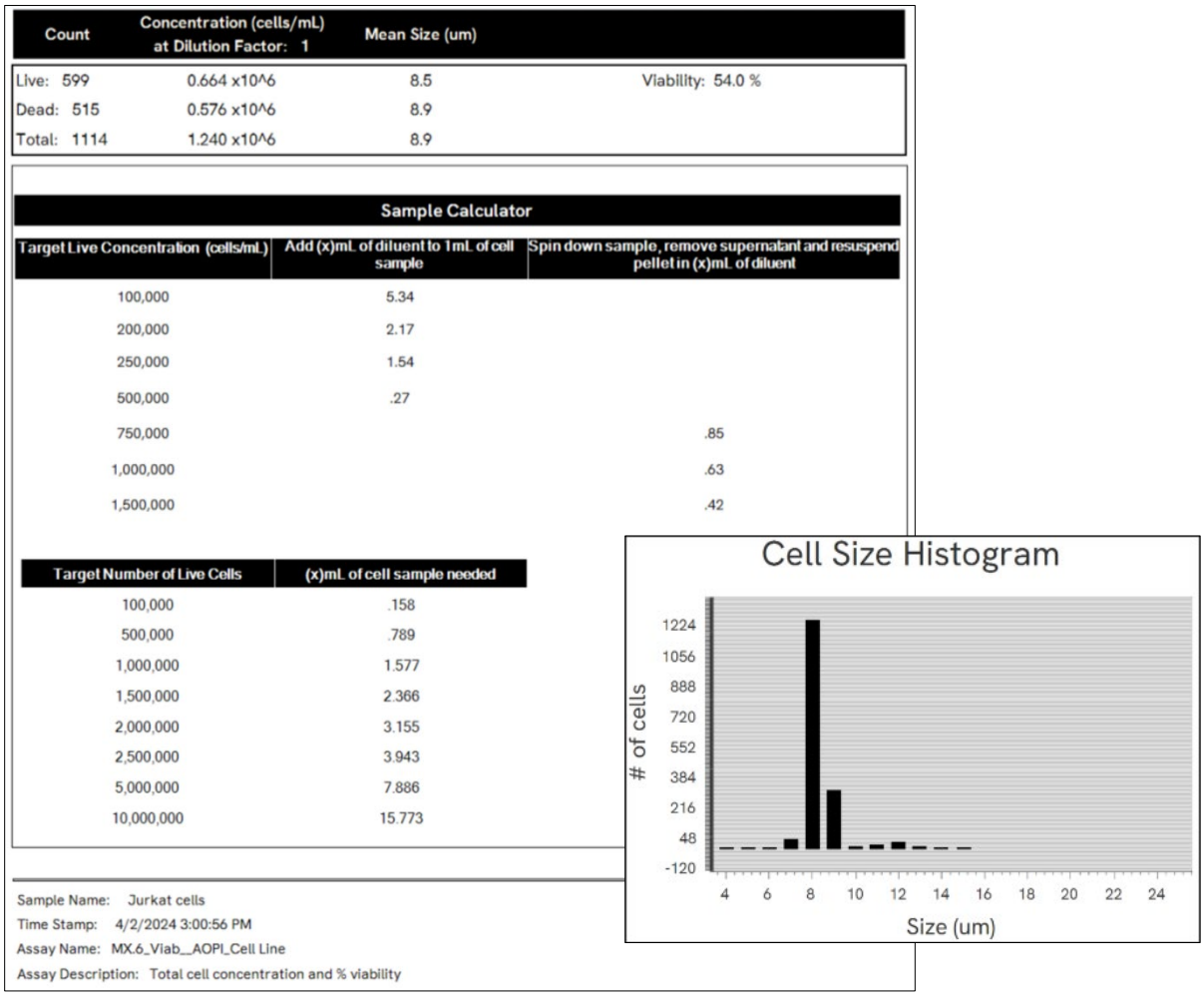
- While using the **Acquire** workflow tab, the Matrix software launches a series of screens that guide you through a sequential process. Use the **Back** button ◀ Back located in bottom left corner of the screen to return to the previous screen in the process. *Clicking the **Acquire** tab while already 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 Select Result 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 will be displayed at the top of the Results List.* Double-click on any scan result in this list to view it. See the ***Matrix Software User Manual*** for details on managing scan results contained in the *Results List*. *Clicking the **Data** tab while already 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, click 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 ***Matrix Software User Manual*** for details on editing assay/cell type parameter settings.

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

- 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 ***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	

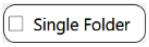
Sample Calculator

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

- 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.*

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Chapter 9. Cleaning, Maintenance and Storage

Keeping the Cellaca PLX 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 Cellaca PLX within a biosafety cabinet, cleaning may not be required, or agents and materials may be adapted according to BSC system requirements. Please follow all 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 be spilled 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 Cellaca PLX. 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 Cellaca PLX 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 an unauthorized personnel attempt to in any way 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 *17*).
- DO NOT put anything heavier than ≥ 25 lbs (11.3 kgs) 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 Power Supply and Power Cord that were 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 Power Switch is in the *ON* position.

Once instrument has been powered on, confirm that multi-colored light oscillating across front of instrument is lit.

Operating Computer cannot connect to instrument

- Check to ensure USB 3.0 Connector Cable that was provided with the instrument is being used.
- Check to ensure USB 3.0 Connector Cable is plugged in properly to both Operating Computer and instrument.
- Reboot Operating Computer to determine if connection issue/error message is resolved.
- If error message persists, reach out to Support for a replacement USB 3.0 Connector Cable or for assistance with re-running the camera software drivers.

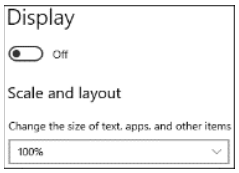
Note: When connecting the computer to the instrument, users must wait until the instrument makes an audible click (i.e., indicating the instrument motors are communicating with the computer) *before* launching the software. Not waiting for this click can result in errors during the startup sequence. *Keep in mind this note will apply each time the computer and instrument are disconnected/connected, or powered off/on again.*

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

Software displays a dark (blank) Preview screen during data acquisition

If the software displays a dark (blank) **Acquire** tab Preview screen during data acquisition, close the software by clicking ***X*** in the upper right corner of the screen.

Right-click on the desktop and select *Display settings*. Scroll down in the right pane of the window displayed to find the *Scale and layout* option. Verify that *100%* (and *not* 125%) is displayed. Re-open the software and preview the sample again.



Software displays an instrument error

The Matrix Software has built-in error messaging that displays when the instrument is operating improperly. In the event that an error persists without resolve, follow these steps *before* contacting Support:

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 *12*.

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

If it is necessary to power down the instrument:

1. Close the Matrix Software window by clicking ***X*** in the upper right corner of the screen.
2. Power OFF the Operating Computer.
3. Power OFF the Cellaca PLX instrument.

FREQUENTLY ASKED QUESTIONS

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

Record serial number of the instrument to request an upgrade. Contact Support by visiting https://www.revvity.com/contact-us or send an email to: CellC-support@revvity.com

How do I order a replacement for an instrument Power Cord or USB 3.0 Connector Cable that has been lost?

Record serial number of the instrument to request a replacement power cord/cable. Contact Support by visiting https://www.revvity.com/contact-us or send an email to: CellC-support@revvity.com

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

Cell types can be imported from either the Revvity Cell Library or from custom libraries stored in external network locations. See the ***Matrix Software User Manual*** for instructions on how to import a cell library and populate the Cell Type drop-down menu with selected cell types. For a full list of available cell types and/or if you require a specific cell type, contact Support by visiting https://www.revvity.com/contact-us or send an email to: CellC-support@revvity.com

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 stain stock solution is being used as indicated in *Trypan Blue Staining Solution Guidelines* on page *25* and *AO/PI Staining Solution Guidelines* on page *27*. Using stains in a concentration *higher* than recommended 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 ***Matrix Software User Manual*** for more information about editing assays and cell type parameter settings.

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Chapter 11. Contacting Support

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

SCOPE OF SUPPORT SERVICES

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

- Online and in-lab customer training
- Creation of new cell types
- Optimization of counting parameters
- 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 or software, contact Support using the following methods:

- Visit https://www.revvity.com/contact-us/instrument-support-and-service and choose the *Cell Counting and Image Cytometry* product for support. Enter instrument serial number, name and model, your contact details, and a detailed description to report the issue to Support.
- Visit https://www.revvity.com/contact-us-by-phone to find the global phone number for your area.
- Send 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 *Chapter 10. Troubleshooting and FAQs* on page *51* 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 *12*.

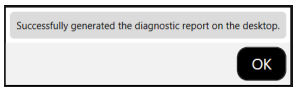
Gathering these details *prior* to contacting Support will be helpful as they troubleshoot the technical issue.

GENERATING DIAGNOSTIC REPORTS

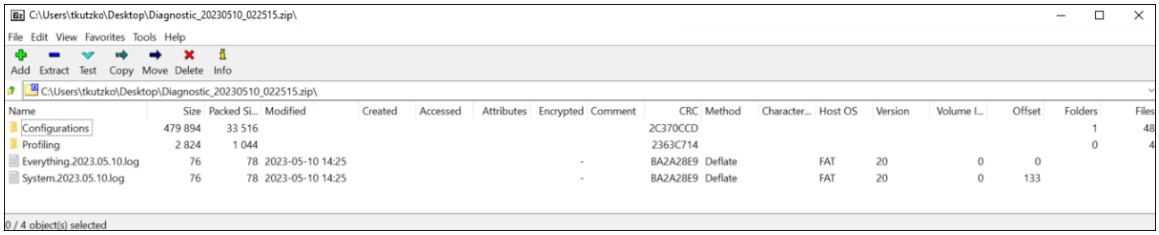
To generate a diagnostic report that can be emailed to Support when experiencing a technical issue:

1. Click the **Home** tab and then the **About Matrix** button.

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



3. From the desktop, click 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\vNNN* where *NNN* represents installed version) and configuration files (located in *C:\ProgramData\Revvity\Matrix\vNNN\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 on a daily basis. Keep in mind that clearing all logs will remove accumulated files to date for the version.*

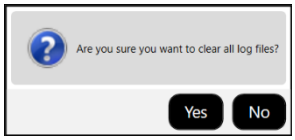
WARNING: Logs may be required to maintain a historical archive. As using the Clear All Logs button will *permanently* remove accumulated logs for the installed Matrix software version, it is recommended that you contact IT *before* clearing logs from your system.

To clear all log:

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

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

2. When prompted, click **Yes** to confirm you want to clear all log files.



3. Click **OK** to acknowledge that all log files have been cleared.

Appendix A. Consumables

This appendix presents Revvity consumables designed specifically for the Cellaca PLX such as disposable counting plates, low fluorescence slides, assay reagents/reagent kits, and counting beads.

COUNTING PLATES AND LOW FLUORESENCE SLIDES

Revvity offers high-throughput *Counting Plates* in *12x2* and *3x8* plate layouts. These disposable, plate-based cell counting chambers are specifically designed for Cellaca instruments.

- Count up to 24 samples in 48 seconds using trypan blue
- Count up to 24 samples in under 3 minutes using AO/PI
- Perform cell-based assays such as apoptosis, mitochondrial membrane potential, fluorescent protein expression (GFP and RFP) with viability, and reactive oxygen species (ROS)
- Low plate-to-plate variability
- Made in the USA to exacting standards with rigorous testing and validation

Visit the *High-Throughput Counting Plates* page on our website for a current listing or contact your Sales representative to purchase counting plates directly from Revvity.

Revvity also offers *Low Fluorescence Slides* to be used with the slide holder included with the instrument (holds up to 4 slides). These disposable, low fluorescence slides are specifically designed for the Cellaca PLX for surface marker detection.

- Image, count, and analyze three fluorescent surface markers in up to 8 samples (full slide holder with 4 slides) in 8 minutes
- Perform cell-based assays such as apoptosis and fluorescent protein expression (RFP and GFP) with viability
- Low slide-to-slide variability

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

ASSAY REAGENTS AND KITS

Revvity offers a variety of *Antibody Immunophenotyping Kits* to accurately perform fluorescence-based cell counting and viability assays, including measuring percent viability and the number of live/dead cells.

Available kits include:

- Single Surface Marker
- Single Isotype Control
- Single Surface Marker Viability
- Two Surface Marker Total Cell
- Two Surface Marker Dead Cell
- Two Surface Marker Viability
- Three Surface Marker Total Cell
- Three Surface Marker Dead Cell
- Apoptosis
- Viability for Fluorescent Protein-expressing 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 Cellaca PLX assay reagents and kits directly from Revvity.

COUNTING BEADS

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

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

Visit the *Counting Beads* page on our website for a current listing or contact your Revvity Sales representative to purchase counting beads for use with Cellaca PLX instruments directly from Revvity.

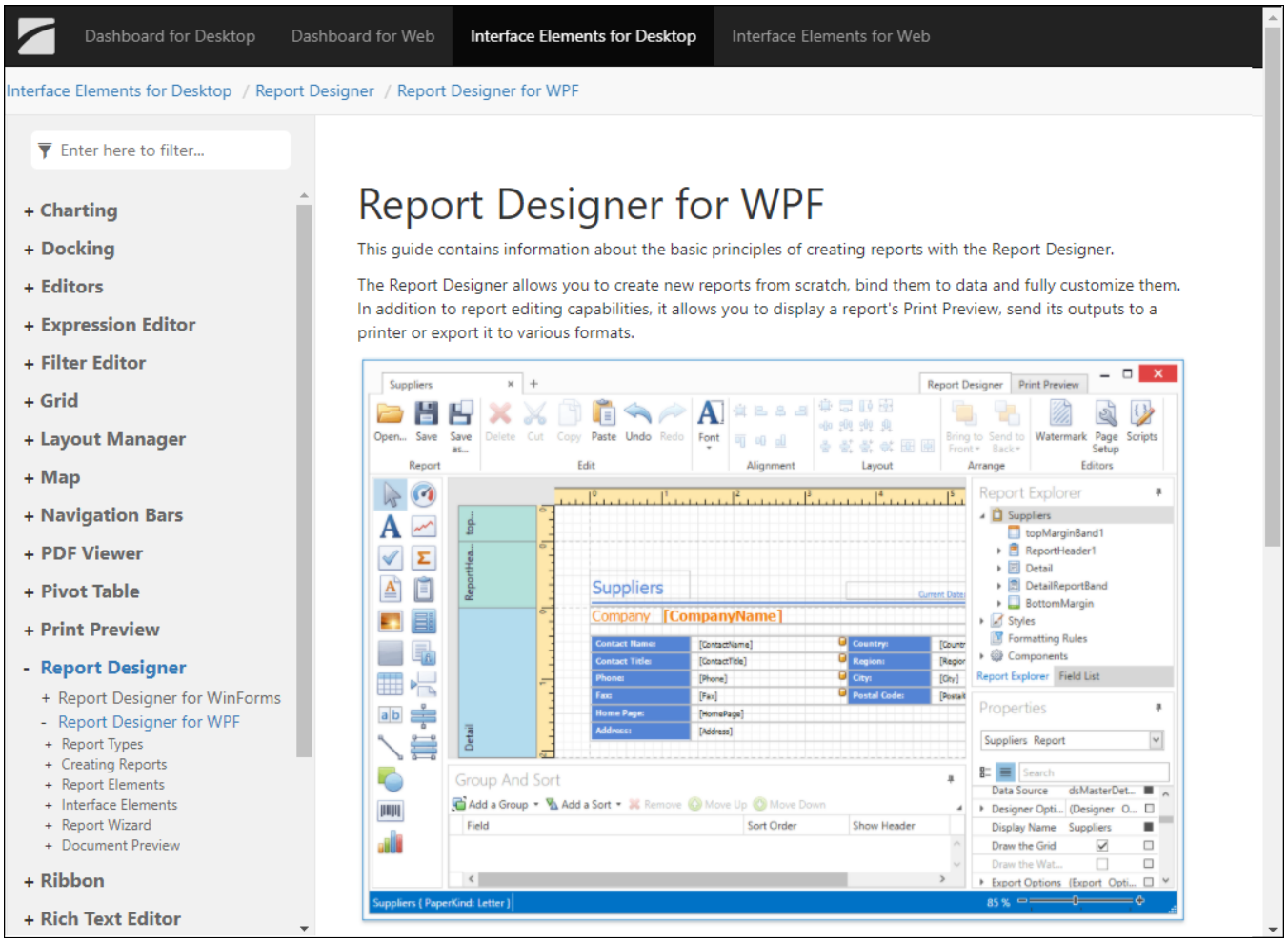
Appendix B. Report Designer for WPF Reference

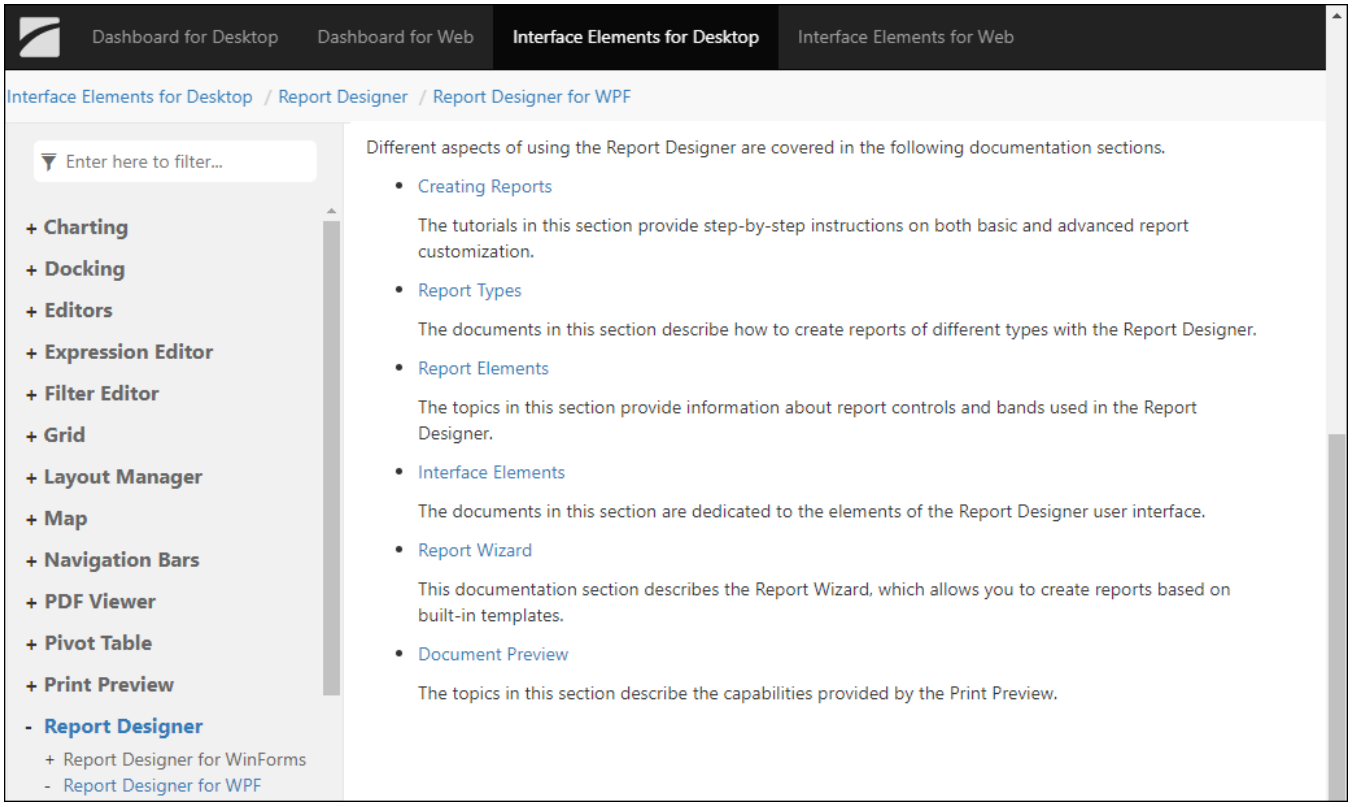
Revvity uses *DevExpress* as a third-party plugin for Cellaca reporting capabilities.

Visit the following page on the DevExpress website for more information about creating Matrix software report templates using *Report Designer for WPF*.

https://devexpress.github.io/dotnet-eud/interface-elements-for-desktop/articles/report-designer/report-designer-for-wpf.html

Sample screens displaying the range of topics available for *Report Designer for WPF* functionality are shown below.




Dashboard for Desktop
Dashboard for Web
Interface Elements for Desktop
Interface Elements for Web
Interface Elements for Desktop / Report Designer / Report Designer for WPF
Enter here to filter...
+ Charting
+ Docking
+ Editors
+ Expression Editor
+ Filter Editor
+ Grid
+ Layout Manager
+ Map
+ Navigation Bars
+ PDF Viewer
+ Pivot Table
+ Print Preview
- Report Designer
+ Report Designer for WinForms
- Report Designer for WPF
Different aspects of using the Report Designer are covered in the following documentation sections.
Creating Reports
The tutorials in this section provide step-by-step instructions on both basic and advanced report customization.
Report Types
The documents in this section describe how to create reports of different types with the Report Designer.
Report Elements
The topics in this section provide information about report controls and bands used in the Report Designer.
Interface Elements
The documents in this section are dedicated to the elements of the Report Designer user interface.
Report Wizard
This documentation section describes the Report Wizard, which allows you to create reports based on built-in templates.
Document Preview
The topics in this section describe the capabilities provided by the Print Preview.

Appendix C. Warranty and License Details

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

WARRANTY INFORMATION

Revvity warrants that Cellaca PLX 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 Matrix software and any subsequent software upgrades is protected. You may not tamper with this software, disclose it to third parties or use it for any purpose other than running your Cellaca PLX system. Revvity, Inc. does not grant you any other rights to use or disclose the original Matrix software or subsequent upgrades, and any further uses will be prosecuted by Revvity to the maximum extent possible by law. Any other use of Matrix software or upgrades is explicitly prohibited. In addition, you may not disclose Matrix software, upgrades, or any of its features and benefits to a third party.

Revvity Proprietary Information

Cellaca PLX products have been developed by Revvity, Inc. 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, Inc. 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)

Cellaca PLX High Speed Cell Counter 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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