Contents of Shipping Container

☐ **Cellaca MX Instrument**

☐ Instrument **Power Supply** and **Power Cord**

☐ **USB 3.0 Connector Cable**

☐ **Revvity-provided Laptop** with **Power Supply** and **Power Cord**

☐ **Matrix Software** (pre-installed on Laptop)

☐ **Cellaca MX User Manual** (PDF file on Laptop)

☐ **Matrix Software User Manual** (PDF file on Laptop)

☐ **Cellaca MX Quick Start Guide** (PDF file on Laptop – *this document*)

☐ **Cellaca MX/PLX Plate Loading Template** – Indicates mixing/loading wells for both 12x2 and 3x8 plate layouts

☐ **Cellaca MX/PLX Focus Guide** – Assists with adjusting instrument focus

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

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

☐ Two **AO/PI Viability Reagent Vials** (**CS2-016-5mL**)

Unboxing the Instrument

Unpack and visually inspect the MX to ensure no physical damage has occurred during shipping. For assistance in setting up the instrument, visit the Cellaca MX page on our website for training videos on unboxing and getting started.

As the MX instrument is run using the *Matrix Software*, the logo distinguishes it from earlier versions.

Powered By **Matrix™**

Site Preparation

Instrument must be placed on a level surface and plugged directly into an electrical outlet. *Use of a surge protector is recommended.* Ensure all cables are free from tangles *prior* to starting the Cellaca MX.

Follow all equipment safety protocols and keep the area around instrument clean both during and post operation. Do *not* position the device so that it is difficult to disconnect from power main.

Setting Up the System

The *Cellaca MX System* is comprised of the instrument connected via USB cable to an Operating Computer (Laptop) that is used to run the Matrix software. The Operating Computer can be linked to a network for accessing external files, printers and for storing scan results.

As the Operating Computer provided by Revvity is a touch-screen device, you can interact directly with the Matrix software GUI by tapping gently on-screen elements (e.g., tabs, drop-downs or buttons) using a finger or stylus. *If you choose to use touch-screen functionality, the term "click" as it appears in this guide may be replaced with "tap" interchangeably.* As an alternative, a USB mouse may also be connected.

Connect instrument Power Supply/Power Cord to the Cellaca MX and plug Power Cord into electrical outlet. Turn MX Power Switch *ON* and confirm that light bar on front of instrument is lit. Set up and power on Revvity-provided Laptop and connect to instrument using 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.*

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

## Calibrating Instrument Prior to First Use

After initial setup of an instrument, you must calibrate it using the Matrix software prior to first-time use. 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 loaded in the instrument. Contact Support for assistance with calibration.

Matrix Screen Elements

Upon launching the Matrix Software you are presented with the **Acquire** tab > *Setup* screen by default. Basic screen elements are described below.

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

The *Navigation Bar* visible across top of the screen is always displayed and displays workflow tabs. It also contains **Eject**/**Load** buttons that control movement of the instrument stage (for unloading/loading consumables).
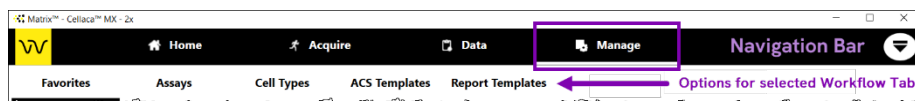
Functionality associated with each workflow tab is described below.

Home Tab: Displays the Matrix logo distinguishing the software from prior versions. Also contains the **About Matrix** button which displays version details and Revvity contact information.

Acquire Tab: Contains the two sequential screens in the *Data Acquisition* workflow. Users can select a *Favorite* (with all assay parameter settings pre-defined) to streamline the workflow or enter setup details before advancing to **Preview** or **Count** (if *Skip Preview* feature is enabled) the sample.

Data Tab: Contains the three sequential screens in the *Data Analysis* workflow. Users must first *Select* a scan result before viewing its count *Results*. You can also perform a *Recount* by fine-tuning assay parameter settings to be used in the analysis to create a new scan result.

Manage Tab: Contains the various system lists of *Favorites*, *Assays*, *Cell Types*, *ACS Templates* and *Report Templates* used during *Data Acquisition* and *Data Analysis* workflows. You can manage system lists by importing/exporting, renaming, deleting or showing/hiding individual entities. In addition, you can create new or modify existing favorites, assays, cell types, and report templates.

See the *Matrix Software User Manual* for full details on using tab functions.

Preparing Plates with Samples

Although the Cellaca MX does not require any routine testing or calibration, counting beads are available to verify instrument functionality. *Revvity counting beads CCBM-011-2ML are recommended for use with Cellaca MX.*

To prepare plates containing counting beads or cell samples:

1. From desktop of Operating Computer, launch the Matrix software by double-clicking the **Matrix** icon.

2. 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.*

3. 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 template graphic, aligning the notched corner of the plate. *You should be able to view and identify the mixing/loading wells through the plate.*

4. Set pipette to 50 µL and then pipette bead solution/ cell sample up and down 10x to break up 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.*
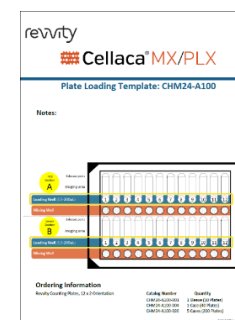
If cell samples require mixing prior to loading, pipette samples into *Mixing Wells* on the counting plate (adjusting volume as necessary to account for dilution factor). Once mixing of samples is complete, pipette 50 µL from *Mixing Wells* into associated *Loading Wells*.

Placing Plate onto Instrument Stage

1. In the Matrix software, click the **Eject** button located in the Header Bar to eject stage from instrument.

2. Place prepared counting plate onto 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 stage).
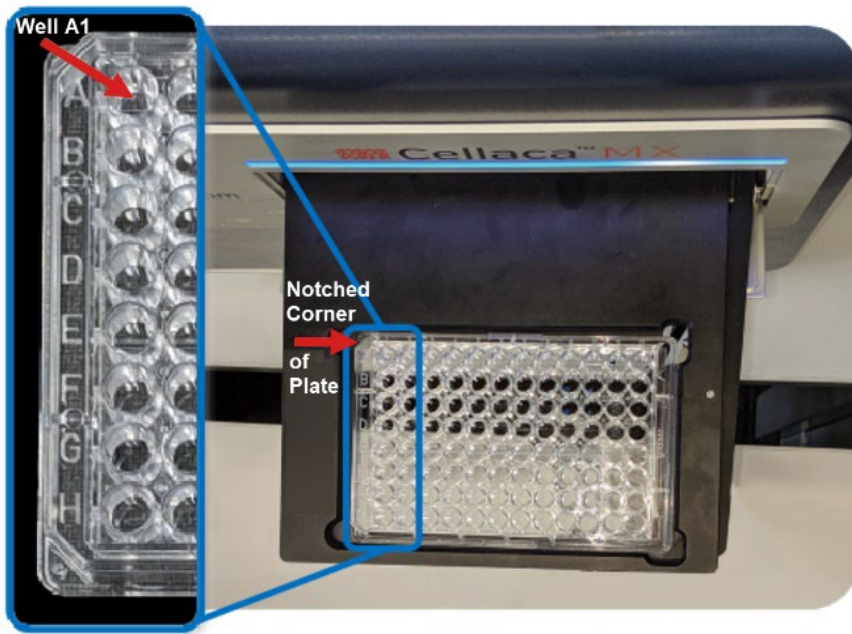
3. Click the **Load** button to retract stage into instrument.

Performing Image Clipping Check

1. Click the **Acquire** tab and select the *Brightfield Channel Verification* assay.

2. Select a well in a corner of the plate and click the **Preview** button.

3. Visually verify that no clipping has occurred around edges of the image.

Bad Image	*Bad Image*	*Bad Image*	*Good Image*
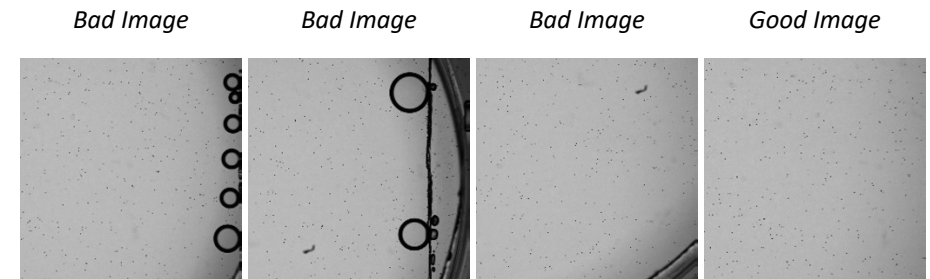
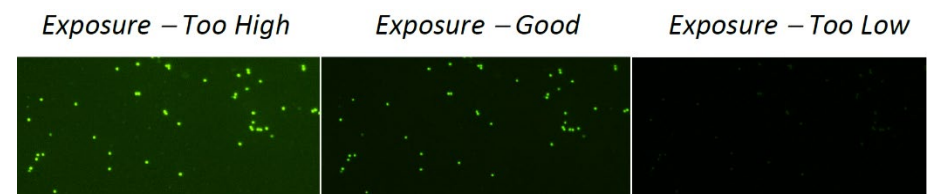4. Repeat this step for each well in remaining outer corners of the plate.

Understanding Instrument Focus

To adjust the focus of a live image being previewed, an **Auto Focus** button allows the instrument to determine the best focal position for selected well. *Focus* controls are also available if additional fine tuning is required.

Live cells should have a bright center and dark, crisp clearly defined edges.

Focus – Too Dark	*Focus – Good*	*Focus – Too Fuzzy*
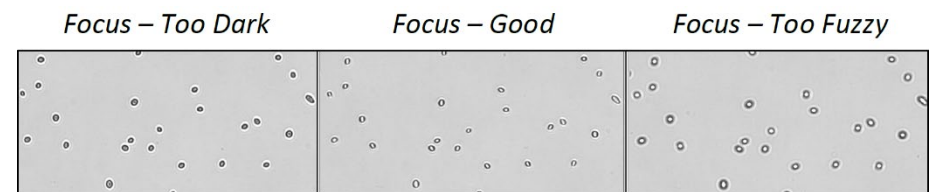
Fluorescent signals should be strong with a low, dark background.

Exposure – Too High	*Exposure – Good*	*Exposure – Too Low*

Counting and Analysis Workflow

Performing a cell count consists of either selecting a *Favorite* or entering *Setup Details*. Upon launch of the Matrix software, the data acquisition workflow is launched by default, starting with the *Setup* screen.
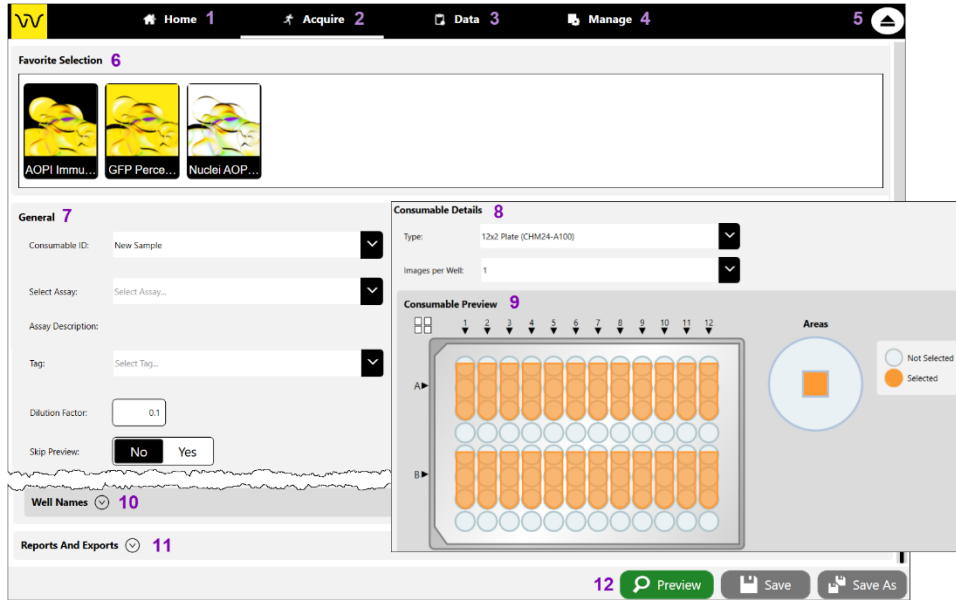
1   **Home Tab** – Displays Home screen with instrument/software details.

2   **Acquire Tab** – Use to enter *Setup Details* and *Preview* live sample images.

3   **Data Tab** – Use to view current scans/select saved scans for re-analysis.

4   **Manage Tab** – Displays assay, cell type and ACS/report template lists.

5   **Eject Button** – Controls instrument stage (toggles between **Eject/Load**).

6   **Favorites Selection Area** – Displays user *Favorites* (i.e., collections of count parameter settings saved for quick re-use).

7   **General Area** – Use to enter consumable ID, select an assay, add a tag, specify a dilution factor or and choose whether to *Skip Preview* screen.

8   **Consumable Details Area** – Use to enter plate type.

9   **Consumable Preview Area** – Displays a Well Map for selecting wells.

10   **Well Names Area** – Use to enter or import names for selected wells.

11   **Reports and Exports Area** – Use to specify auto export location.

12   **Preview Button** – If plate is loaded, *Setup* details are entered and a well is selected, displays live images to adjust focus/fluorescence exposure.

Entering Setup Details

1. In the **Acquire** tab, *Setup* screen, enter a **Consumable ID**. *If you do not enter a consumable ID, 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. The **View** button is available if you find it necessary to edit assay details prior to previewing the live image.

3. 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.

4. Select consumable **Type** (e.g., *12x2* or *8x3* plate layouts) and in the *Consumable Preview* area, click on wells containing samples. 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 the block and hold button down while dragging mouse to the end of the block before releasing button. To select or de-select *all* wells, click the **All Wells** ⊞ button.

5. If desired, expand the *Well Names* area to enter/import well names or the *Reports and Exports* area to modify the default auto export location.

6. Click the **Preview** (if enabled) or **Count** button.

Previewing Live Images

The Preview screen displays live images for selected wells.

1. Expand the *Details* area to view selected assay and Consumable ID.

2. In the *Consumable* area, click on highlighted wells to view live images of samples in the plate. As you move from well to well, the live image displayed will change accordingly.

To zoom in and out of an image, move the mouse to hover cursor over the viewing pane and turn the scroll wheel or, if using the touch screen, apply universal gestures (e.g.,
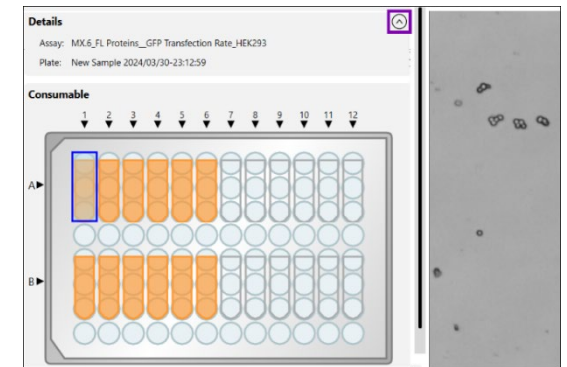
touch the center of the image with two fingers and then slowly spread them apart to zoom in and reverse this action to zoom out.) *Zoom magnification is displayed in bottom right corner of the viewing pane.* To move an image around, click on the image and drag to a new location.

3. Adjust focus of the image using focusing controls. *The Channel 1: Brightfield image is displayed by default.* Click the **Auto Focus** button or use the manual *Focus* controls if additional fine tuning is required.
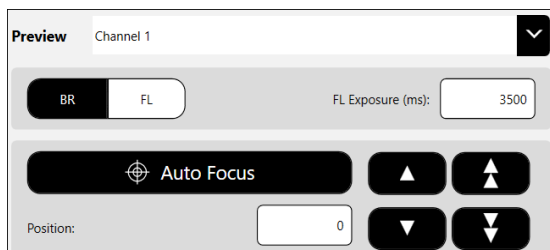
Auto Focus	Allows instrument to determine the best focal position for the selected well.
Focus Position	Allows users to enter a numerical value for vertical (Z) position of the objective lens.
Fine Focus Manual Offset	Allows users to finely adjust vertical (Z) position of the objective lens for optimal focus (in µm). Click the up/down single arrows to adjust focus.
Coarse Focus Manual Offset	Allows users to coarsely adjust vertical (Z) position of the objective lens for optimal focus (in µm). Click the up/down double arrows to adjust focus.

Note: For BR/FL imaging modes, each channel will be associated with two images – *Brightfield* (*BR*) and *Fluorescent* (*FL*). 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*.

4. In the **Preview** field, click the drop-down to view available channels. *Channels displayed will depend on assay Imaging Mode.* Select a channel to change image display.

5. For BR/FL imaging modes, click **BR** or **FL** buttons to change image display. *BR image will always be displayed by default.* Adjust **FL Exposure** value for FL images associated with each channel, if needed.
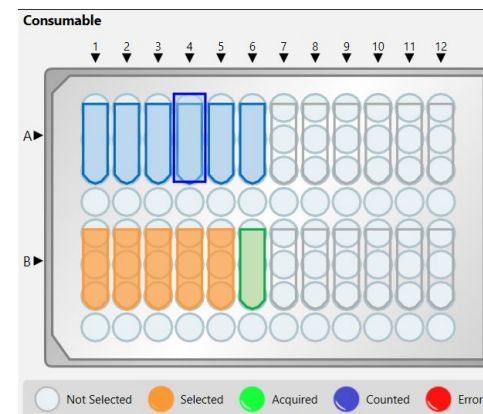
6. Click the **Count** button.

Analyzing Scan Results

As the system acquires sample images, the colors used to mark selected wells change to indicate well status (i.e., from *Selected* to *Acquired* to *Counted*) as shown in the legend displayed below the Well Map.

You can click on a well as soon as it is *Counted* to display its image (in the viewing pane) and its count results (below the viewing pane).

Well images are displayed in the **Well View** tab along with acquired data for the selected well. Additional tabs may be enabled for the scan result (e.g., **Summary** and **.csv**).


Scan results are displayed (using assigned report tabs/templates), printed and exported based on *Reports and Exports* options defined for the assay.

When analyzing scan results, an image is displayed for the selected well. To view images from other wells, click on each well in the Well Map.

1. In the *Consumable* area, click on a well to display its image in the viewing pane.

2. At the top of the **Well View** tab, click the *Brightfield* (**BR**) or *Fluorescence* (e.g., **FL 1**, **FL2**, etc.) buttons to select/de-select channels used in display of the image. *Channel views are overlaid on top of each other.*

3. Click the **Counted Overlay** button to show/hide a graphic overlay that highlights *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 specified cell diameter). For Expression assays, outlining is used for total cells in the masked channel but is only displayed in one color – Blue.*

4. Click the **Zoom** button to enable/disable display of *Zoom* magnification in bottom right corner of viewing pane. *Zoom feature will still be functional even if not displayed.*

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

6. Click the **Summary** tab (displayed for a Matrix v4.0+ assay) or **All Wells** tab (displayed for a v3.0 assay) to view a full page containing count results for all scanned wells.

Verifying Auto Exports

Expand the *Exports* area in the Results screen to verify that automatic exports were completed and click the location link to view exported scan result files. If any output file types (e.g., *CSV*, *Excel*, *PDF* and *Word*) were selected to be generated for assay reports, verify that file type exports were also completed.

To modify default *Reports and Exports* options defined for an assay during a count/recount, click the **View** button associated with the selected assay and expand the *Reports and Exports* area for the assay. You can either save your changes to the assay (i.e., any changes will be applied to other scan results that use the assay) or save the changed assay as a copy with a new name and then select it to be used for data acquisition.

An alternative to modifying default *Reports and Exports* options for an assay is to perform a manual export *after* image acquisition by clicking the **Export** button at the bottom of the Results screen and selecting additional options.

Performing a Recount

If you find it necessary to fine-tune assay parameters after analyzing scan results, click the **Recount** button located at bottom of the Results screen.

View parameter settings for the *Last Used Assay* or select a new *Assay for Recount* and view/edit its parameters.

You can de-select wells to exclude them from the recount, but you cannot add wells for the recount that were not included in the initial count.

To manage channel mappings in a recount, expand the *Channel Mappings* area. 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.

Viewing the Data Tab

The **Data** workflow tab launches the *Select*, *Results* and *Recount* screens which must be completed in sequence when analyzing scans.
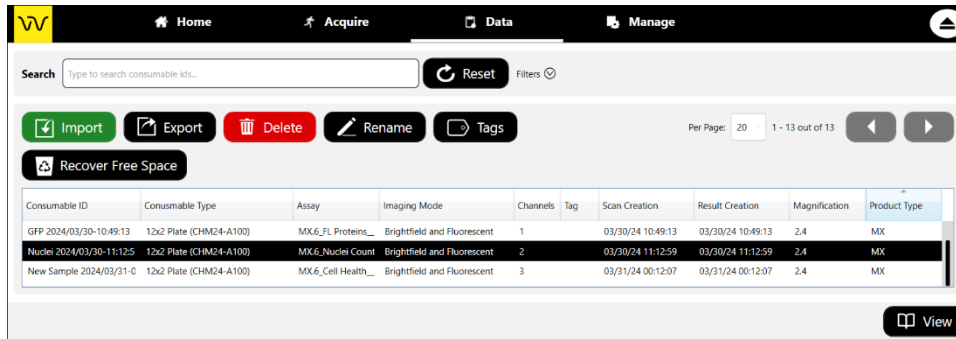
To open a scan result contained in the *Results List* of the Select screen you can either double-click the result or click it once (to highlight it in the list) and then click the **View** button.

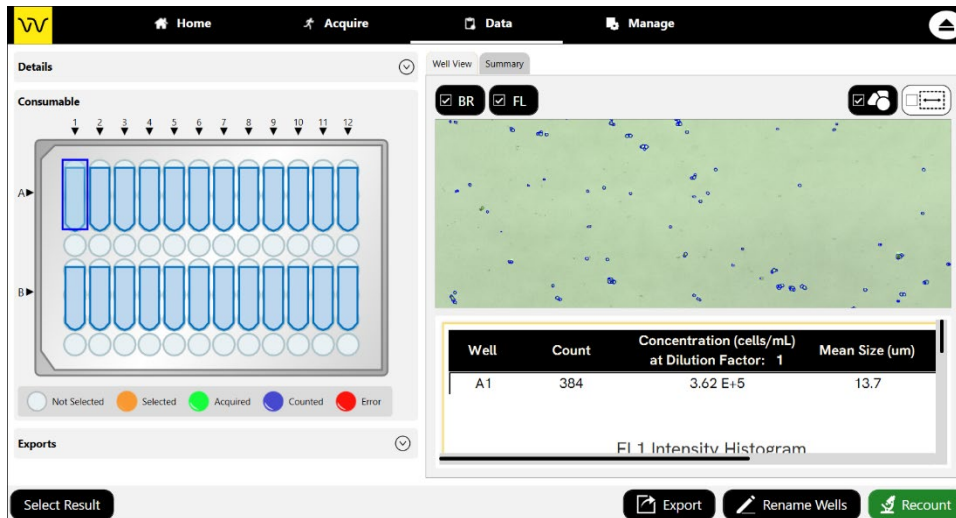Once a scan result is displayed vary images in the **Well View** tab by changing wells and selecting channels or click the **Summary** tab to view count results.

Viewing the Manage Tab

The **Manage** tab lists *Favorites*, *Assays*, *Cell Types*, *ACS Templates* and *Report Templates* loaded on your instrument system. From this screen you can import/export, rename, delete or show/hide any of these entities in their respective lists.

The *State* column displays icons indicating whether an entity is *Locked* or *Unlocked*. Although a locked assay, cell type or report template cannot be edited, you can select it to use as a source for creating a new entity and click the **Save As** button to save it using a new name.
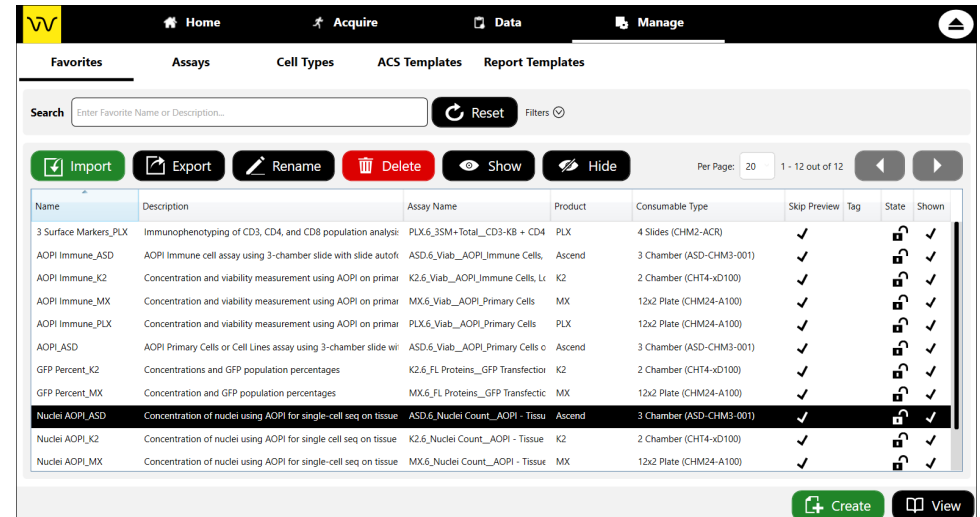
ACS Templates can be imported on to your system via the Matrix software. You can assign an ACS template to an assay if the *Object Level ACS* export and *Use Template* options are selected. On export, data will auto populate into the specified template and be saved as a file. *If the Auto Open option is also selected and the De Novo Software FCS Express application is installed on your system, FCS Express will be launched for viewing export data.*

Revvity provides extensive assay, cell type and report template libraries as defaults in the Matrix software. Contact Support for current listings of these libraries or for help with creating new assays, customizing cell type parameter settings and defining new cell types. For assistance with report templates, share with us the goal you are trying to accomplish and we will create a new template to accommodate your needs.

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

If comparing the *Trypan Blue* and *AO/PI* methods, a portion of the sample should be stained with trypan blue and another portion stained with AO/PI.

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

Using AO/PI Viability

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

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

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

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

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 solution by pipetting up and down 10x before adding sample to counting plate loading wells.

The table below shows recommended dilutions when prepping cell samples for AO/PI viability and final *Dilution Factor* to enter for a few sample types.

Sample Type	Preliminary 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

Viability Staining Solution Guidelines

Use the following staining solution guidelines to prepare cell samples.

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

Available Product Documentation

See the following documentation for additional instrument information:

- *8002958 Cellaca MX User Manual* for instrument operation, care and maintenance details (available as a PDF on desktop of the Revvity-provided Laptop shipped with instrument).

- *8003394 Matrix Software User Manual* for complete details on using software functionality (available as a PDF on desktop of Revvity-provided Laptop shipped with instrument).

- *8002871 Cellaca MX/PLX Plate Loading Template* for use when loading CHM24-A100 (*12x2*) and CHM24-B100 (*3x8*) Revvity counting plates.

- *8002845 Cellaca MX/PLX Focus Guide* for assistance with optimal focus.

Contacting Support

If there is a technical issue with your instrument, contact Support by visiting https://www.revvity.com/contact-us/instrument-support-and-service or by sending email to: CellC-support@revvity.com

Trained specialists are available to assist your team with sample analysis and optimization of assay/cell type imaging parameters.

When reporting a technical issue, it is recommended that you record any error messages generated, the sequence of steps leading up to the error, and the Serial Number of the instrument *prior* to contacting Support.