Cellaca® MX Quick Start Guide

8002846 Rev D

Contents of Shipping Container

- ☐ **Cellaca MX Instrument**
- ☐ **Power Supply** and **Power Cord**
- ☐ **USB 3.0 Connector Cable**
- ☐ **Nexcelom-provided Laptop** with **Matrix Software** (*pre-installed*)
- ☐ **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 Plate Loading Template** – Graphic sheet indicating mixing/loading wells for both 12x2 and 8x3 plate layouts
- ☐ **Focus Guide** – Graphic sheet to help with adjusting instrument focus
- ☐ **Nexcelom Counting Plates** (24-well, 12x2 layout)
- ☐ **AO/PI Viability Reagent**
- ☐ **Fluorescent Beads**

Unboxing the Instrument

Unpack and visually inspect the Cellaca MX to ensure no damage has occurred during shipping. For assistance in setting up the instrument, visit the Cellaca MX page on our website, click the **Resources** tab and scroll down to find training *Videos* on unboxing and getting started.

As the Cellometer 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 a surge protector (recommended) or power outlet. 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

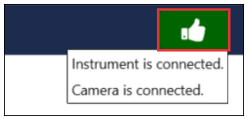
The *Cellaca MX System* is comprised of the instrument connected via USB cable to an Operating Computer that is used to run the Matrix software. The Operating Computer can be linked to a network for accessing external files, printers and for storing count results.

As the Operating Computer provided by Nexcelom 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 the Power Supply/Power Cord to instrument and plug Power Cord into electrical outlet. Connect Cellaca to Nexcelom-provided Laptop using USB 3.0 Connector Cable. Turn instrument Power Switch to *ON* position and confirm that light bar appearing across the front is lit.

From 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.

Click the icon located in upper right corner of the screen to confirm that text in the dialog box displayed indicates both the instrument and camera are connected. *Click the icon once again to close the dialog box.*



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Calibrating Your Instrument

After initial setup of an instrument, you must calibrate it using the Matrix software prior to first 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 Nexcelom Support for assistance.

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 *Header Bar* appearing across the top of the screen contains plate **Eject/Load** buttons that control movement of instrument stage (for loading and unloading of counting plates) and instrument status icons. It will also display assay/plate names as you proceed through the count process.

The *Navigation Bar* appearing on left side of the screen is always displayed. Functionality associated with each tab is described below.

Home Displays the Powered By MATRIX logo distinguishing the software from prior versions. Also contains the **About Matrix** button which displays version details and Nexcelom contact information.

Acquire Displays screen in the *Data Acquisition Process* to which you have progressed. For example, when clicking the **Acquire** tab you will enter *Setup* details and then *Preview* the sample to confirm focus and fluorescent exposure.

Data Displays screen in the *Data Analysis Process* to which you have progressed. For example, when clicking the **Data** tab you will *Select* a scan result and then view count *Results*. You can also perform a *Recount* by updating parameter settings used in analyzing the scan to create a new result.

Manage Displays options for customizing assays, cell types and report templates used in the counting/analysis workflow.

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

Staining Solution Guidelines

Use the following staining guidelines to prepare cell samples for Cellaca MX.

Stain Type	*Use with Cell Sample*	*Dilution Factor*
Trypan Blue (0.2%)	1:1	2
A0 (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

See *Evaluating Viability Methods* on page *7* for details on how to choose a staining method based on the cell sample and selected assay type.

Preparing Sample Plates

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

To prepare sample plates containing counting beads or cell samples:

1. From desktop of Operating Computer, launch the Matrix software by double-clicking the **Matrix** software icon.
2. Place the *Cellaca MX 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 corners of the plate. *You should be able to view and identify the mixing/loading wells through the plate.*
3. Invert counting bead solution or tube containing cell sample a total of 10 times. *If using counting beads, vortex bead solution for 10 seconds. Do not vortex cell samples.*

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4. If using bead solution or a cell sample that does *not* need to be mixed, set pipette to 50 µL and then pipette bead solution/cell sample up and down ten times (10x) to break up any potential clumps. Load 50 µL of bead solution into *Loading Well* of counting plate.

   If using a cell sample that needs to be mixed prior to loading, use the *Mixing Well* of counting plate to prepare the sample and then load 50 µL of cell sample into *Loading Well* of counting plate.

   Note: Liquid will move from the *Loading Well* into the *Imaging Area*.

5. From the desktop of Operating Computer, click the **Eject** button located at the top of the **Acquire** tab to open the instrument stage. Place the prepared plate onto the stage taking care to align the notched corners of the plate with the stage accordingly (i.e., well *A1* is in top left corner).
6. Click the **Load** button to close the instrument stage.
7. In the *Setup Details* area of the screen, enter a **Plate Name**, select an appropriate assay from the drop-down and enter a **Dilution Factor**.
8. In the *Well Details* area, ensure the displayed **Plate Type** is correct, then click on the individual wells used to load the sample.

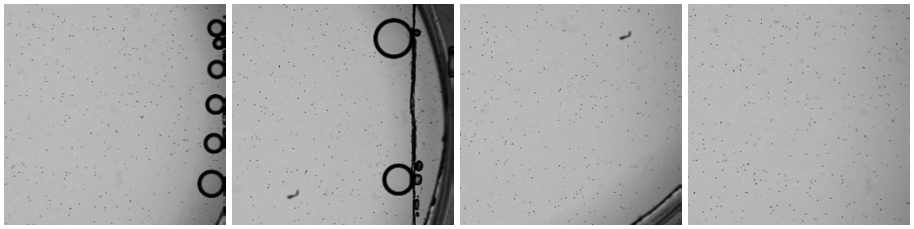
   To select or clear multiple wells, click a well and hold/drag your mouse to encompass other wells. To select or clear all wells, click the ⊞ button.
9. Click the **Preview** button to view the sample.

Note: Cell concentrations of 1.0 x 10^5 – 1.0 x 10^7 cells/mL can be analyzed on the Cellaca MX, with a concentration of 1.0 x 10^6 cells/mL being optimal. Do *not* shake or vortex the sample as this may damage cell membranes.

Performing Image Clipping Check

1. Click the **Acquire** tab and select *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 the edges.
4. Repeat this step for wells in remaining corners of the plate.

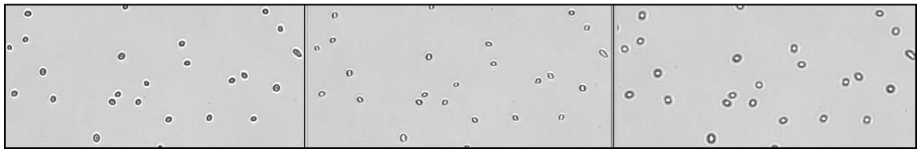
Bad Image *Bad Image* *Bad Image* *Good Image*



Understanding Instrument Focus

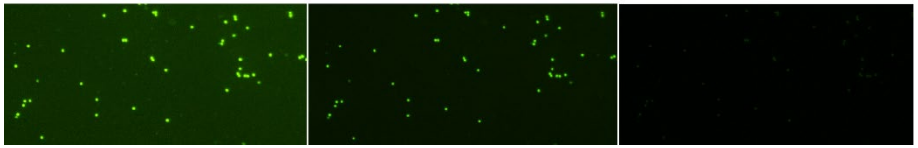
Live cells should have a bright center and dark, crisp clearly defined edges.

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*



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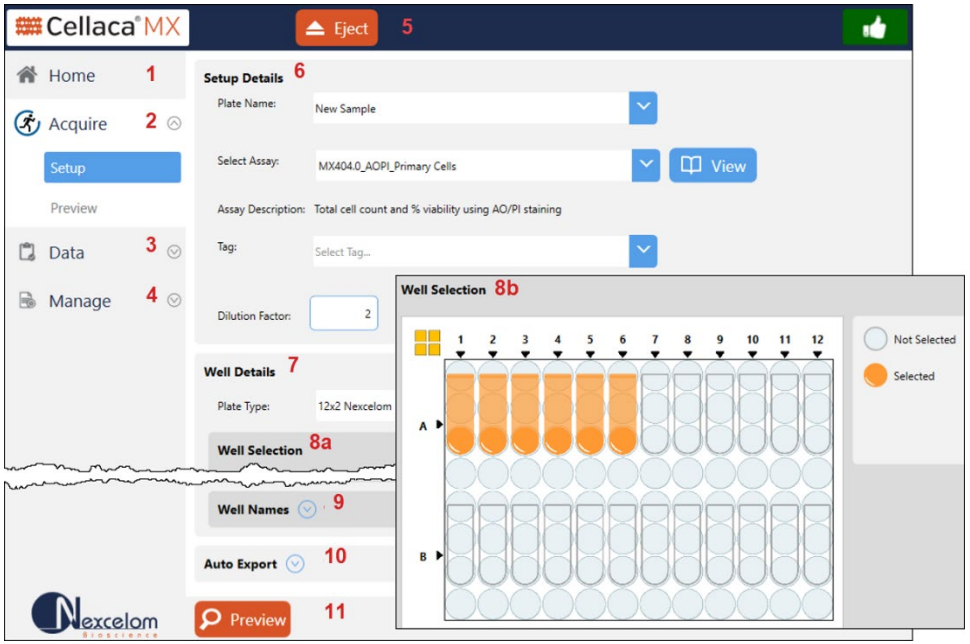
Counting and Analysis Workflow

Performing a cell count consists of *Entering Setup Details* and *Previewing the Sample Image* before *Reviewing Count Results*. Upon launch of the Matrix software, the **Acquire** tab > *Setup* screen is displayed by default.



1 **Home Tab** – Displays Home screen with instrument/software details.
2 **Acquire Tab** – Use to enter *Setup Details* and *Preview* the sample image.
3 **Data Tab** – Use to view current scans/select saved scans for re-analysis.
4 **Manage Tab** – Displays *Assay*, *Cell Type* and *Report Template* libraries.
5 **Eject Button** – Controls instrument stage (toggles between **Eject**/**Load**).
6 **Setup Details** – Use to enter plate name, select an assay, add a tag (to group scan results for custom reporting) and dilution factor.
7 **Well Details** – Use to enter plate type.
8 **Well Selection** – Displays a plate map for use in selecting wells.
9 **Well Names** – Use to define well names for selected wells.
10 **Auto Export Area** – Use to define auto export location and options.
11 **Preview Button** – If plate is loaded and *Setup* details entered, displays sample in *Preview* mode to adjust focus and fluorescence signals.

Entering Setup Details

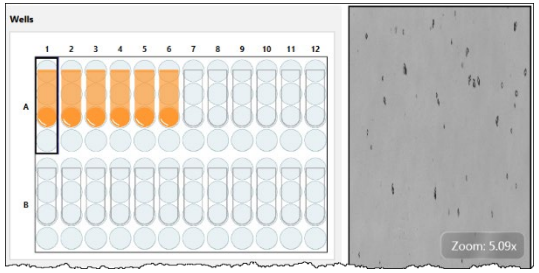
1. In the **Acquire** tab, *Setup* screen, enter a plate name and select an assay. *If you do not enter a plate name, a date/time stamp is appended to the "New Sample" default (e.g., New Sample 2021/03/01-11:58:09).*
2. Select plate type (e.g., *12x2* or *8x3* formats) and click on loaded wells in the plate map. 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 icon.
3. If desired, enter *Auto Export* options including a default export location and image file types for automatically saving scan results.
4. Click the **Preview** button.

Previewing Live Images

The Preview screen displays live images for selected wells. Current *Zoom* magnification is displayed in the bottom right corner of viewing pane.

1. In the Wells area, click on highlighted wells to view live images of samples in the plate. As you move from well to well, the live image being displayed changes per the selected well (i.e., which is indicated in the plate map by an outline surrounding the well).

   To zoom in and out of the image, turn the scroll wheel on your mouse 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.) Current *Zoom* magnification is displayed in bottom right corner of the viewing pane.



   To move around an image, click once inside the viewing pane and hold your mouse button down while dragging the image to a new location.

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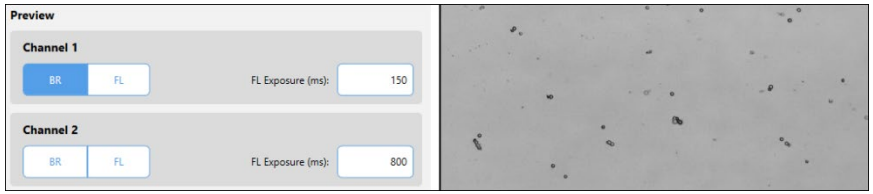
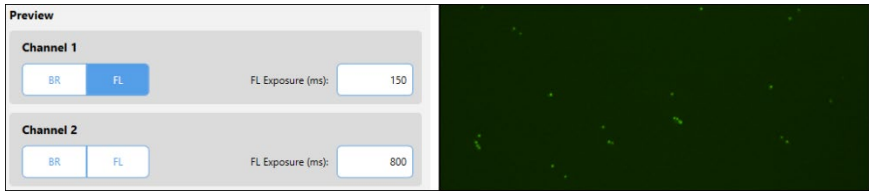
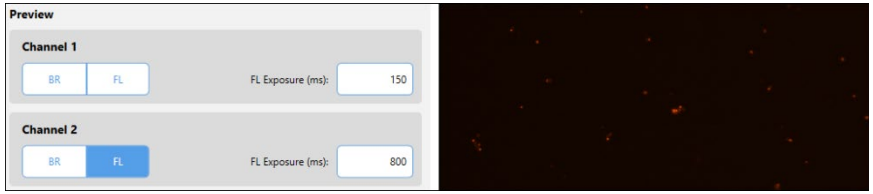
2. In the *Preview* area, the channels displayed for viewing (e.g., *Channel 1|Brightfield* and *Channel 1|Fluorescent,* and *Channel 2|Brightfield* and *Channel 2|Fluorescent*) will depend on configuration of your instrument and *Imaging Mode* of the assay. The *Channel 1|Brightfield* image is displayed by default as shown below.



3. To view other available channels (i.e., channel display is based on selected assay), click the **FL** buttons accordingly. For example, sample *Channel 1|FL* and *Channel 2|FL* images are shown below.

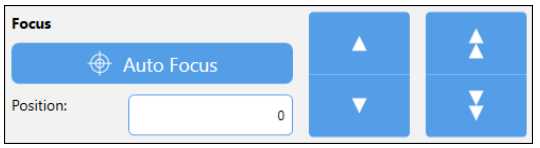




4. Adjust the focus for brightfield image and verify fluorescent exposure.

   Note: If a fluorescent image is available for *Channel 1*, use the *BR 1* image to adjust instrument focus and then select the *FL 1* image to confirm exposure. If a fluorescent image is available for *Channel 2*, you need only to confirm fluorescent exposure. *Keep in mind that a slight offset exists between the BR 1 and BR 2 images due to the distance the camera has to travel between the two filter sets.*

   To adjust focus of the live image being previewed, use the *Focus Controls* as indicated in the following table. Obtaining good focus is key to ensuring accurate cell counts.

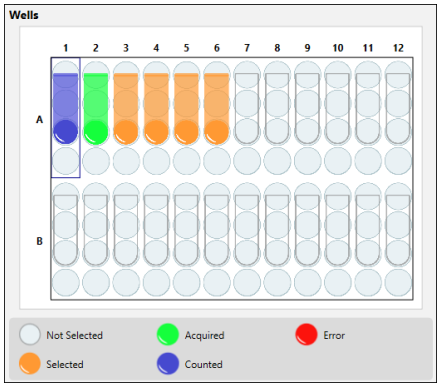


Auto Focus	Allows instrument to determine the best focal position for the selected well.
Position	Allows users to enter a numerical value for vertical (Z) position of the objective lens.
Fine Focus Manual Offset	Allows users to finely adjust vertical (Z) position of the objective lens for optimal focus (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.

5. Click the **Count** button.

Reviewing Count 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 plate map.



As soon as a well is *Counted*, you can click on that well to display its results below the viewing pane. *Count results are displayed in the **Well View** tab using the assigned Display report template.*

Additional tabs may be enabled for the scan result (e.g., **Summary** and **.csv** tabs for Matrix v4.0; **All Wells** tab for v3.0). *Count results will be displayed, printed and exported based on Reporting options defined for the assay.*

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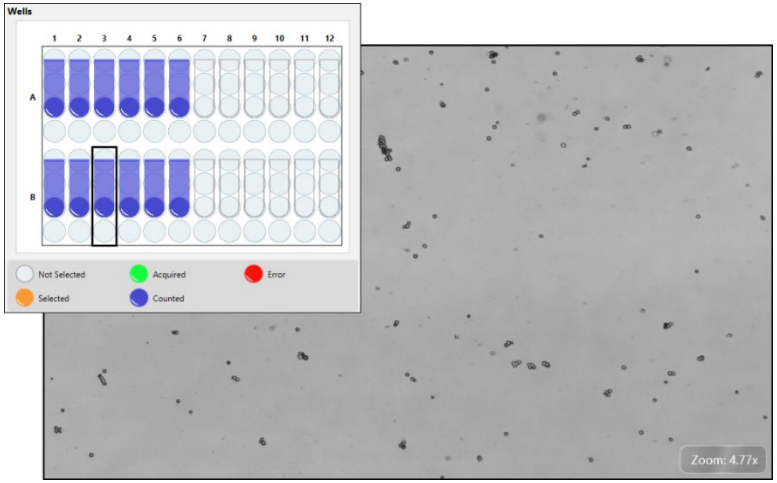
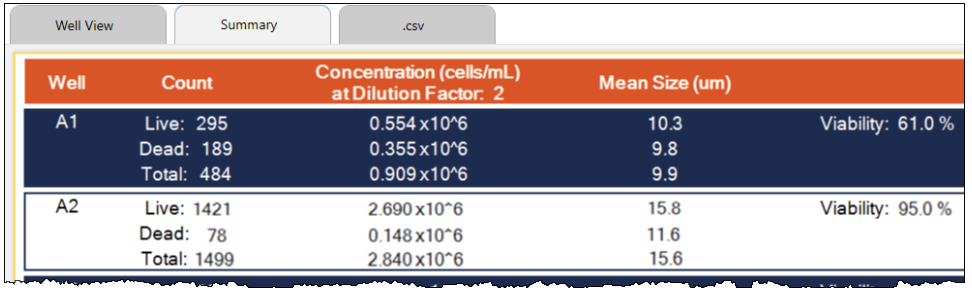
When viewing count results, a single image is displayed representing the sample in the outlined well. Click on other highlighted wells to view them.



1. In the *Wells* 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**), *Fluorescence* (**FL1**) or *Dual Fluorescence* buttons (**FL2**) to change the presentation of the image. *Channel views are overlaid on top of each other.*
3. Click the **Zoom** button to enable/disable display of the current *Zoom* magnification in the bottom right corner of viewing pane.
4. Click the **Counted** button to show/hide a graphic overlay that highlights *Counted* cells by circling them with color-coded outlines (e.g., *Green* for counted/live cells, *Red* for dead cells and *Yellow* for cells not counted because they were larger than the specified cell diameter).
5. At the bottom of the **Well View** tab is a report containing well-level details for the sample image in the selected well.

Well	Count	Concentration (cells/mL) at Dilution Factor: 2	Mean Size (um)	
A1	Live: 1431	2.700 x10^6	15.3	Viability: 94.0 %
	Dead: 86	0.163 x10^6	10.6	
	Total: 1517	2.860 x10^6	15.1	

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 selected wells.



Well	Count	Concentration (cells/mL) at Dilution Factor: 2	Mean Size (um)	
A1	Live: 295	0.554 x10^6	10.3	Viability: 61.0 %
	Dead: 189	0.355 x10^6	9.8	
	Total: 484	0.909 x10^6	9.9	
A2	Live: 1421	2.690 x10^6	15.8	Viability: 95.0 %
	Dead: 78	0.148 x10^6	11.6	
	Total: 1499	2.840 x10^6	15.6	

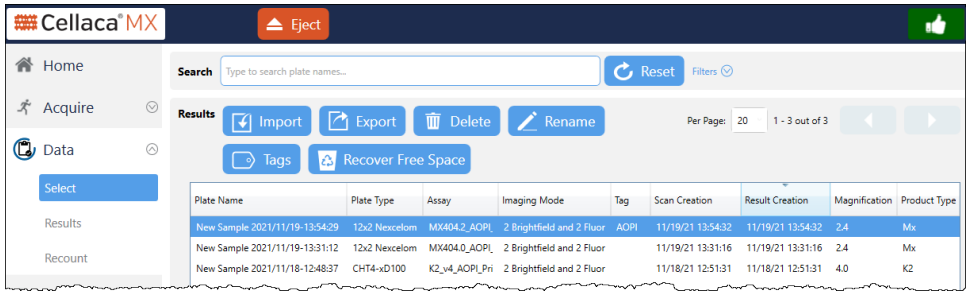
Exporting/Printing Scan Result Data

To update export options for scan result files (*Auto Export* options may be defined in the Setup Screen) as well as custom report files, click the **Export** button located at the bottom of the Results screen.

1. Confirm the current export location. Click **Browse**, navigate to a folder on your Operating Computer or network and click **OK**.
2. Select the scan result files (e.g., *Raw Images*, *Colorized Images*, *Data Set*) to be exported. *Data sets contain all images, results, assays, cell types and report templates associated with the scan result and are stored as .SCANRESULT files.*
3. For reports, select file types (i.e., *Excel*, *CSV*, *PDF*, *Word*) to be exported and if files are to be automatically opened/printed upon export.
4. Click the **Export and Print** button.

Viewing the Data Tab

The **Data** tab contains the *Select*, *Results* and *Recount* screens which must be completed in sequence when analyzing scan results (i.e., you must select a scan result before you can view its data and then fine-tune assay/cell type parameter settings before you can perform a recount).



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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.

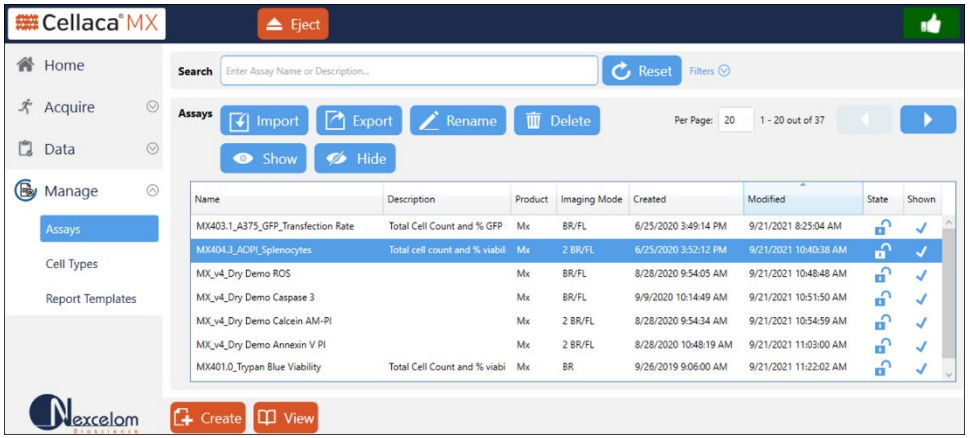
Once a scan result is displayed you can analyze data by clicking channels and varying the presentation of images displayed. To fine-tune assay parameter settings, click the **Recount** button. *If it becomes necessary to select another scan result while you are in the Results screen, click the Select option in the Navigation Bar to choose another scan result.*

To fine-tune assay parameters for the scan result, you can click the **View** button for either the *Last Used* assay or a selected new assay, and edit its parameters as necessary. To perform a recount, click the **Recount** button.

As you navigate through each screen, the *Navigation Bar* is automatically updated a to reflect your progression. After a recount is performed, the Navigation Bar returns to the **Data** tab > *Results* screen.

Viewing the Manage Tab

The **Manage** tab lists all *Assays*, *Cell Types* and *Report Templates* loaded in your 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 that identify the current state of an entity indicating whether they are *Locked*, *Unlocked* or a *Nexcelom System* default. Although a locked/system 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.

Nexcelom provides extensive assay, cell type and report template libraries as defaults in the Matrix software. If you would like help creating a new assay, customizing cell type parameter settings for an assay, or creating a new cell type, send saved raw images to Nexcelom Support and we will optimize a custom assay/cell type for you. For help with customizing report templates, share with us the goal you are trying to accomplish and we will create a new template to accommodate your needs.

For a complete list of available assays, cell types and report templates, contact Nexcelom Support.

Evaluating Viability Methods

When evaluating viability methods, it is critically important to use a single aliquot from the stock cell culture to perform *all* testing. The cell sample should be evaluated for concentration on the Auto 2000 prior to staining.

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.

Using Trypan Blue Viability Method

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

Preparing Cell Sample for Trypan Blue Viability Determination

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.

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.

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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*, while propidium iodide (PI) enters only dead cells with compromised membranes and stains their DNA causing dead nucleated cells to fluoresce *Red*.

- 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, there is no interference from debris and non-nucleated cells when using the AO/PI viability method.

Preparing Cell Sample for AO/PI Viability Determination

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.

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

Invert the tube containing cells ten times (10x) and pipette up and down 10x to generate a homogeneous cell sample and reduce cell clumps. *Vortexing may help for some samples, but may generate bubbles that make it difficult to pipette when working with small sample volumes.*

If necessary, dilute original sample with PBS. 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 prior to transfer. Gently mix by pipetting up and down 10x before adding sample to counting plate wells.

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

Sample Type	*Preliminary Sample Dilution (with PBS)*	*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

Contacting Nexcelom Support

Nexcelom Support is available from 8:30 am to 5:30 pm EST and can be reached at +1 (978) 327-5340 or by sending email: **support@nexcelom.com**

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

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 Nexcelom-provided Laptop shipped with your instrument).
- ***8003394 Matrix Software User Manual*** for complete details on using software functionality (available as a PDF on desktop of Nexcelom-provided Laptop shipped with your instrument).
- ***8002845 Cellaca MX Focus Guide*** for assistance with optimal focus.

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