



Cellaca MX High Throughput Automated Cell Counter User Guide

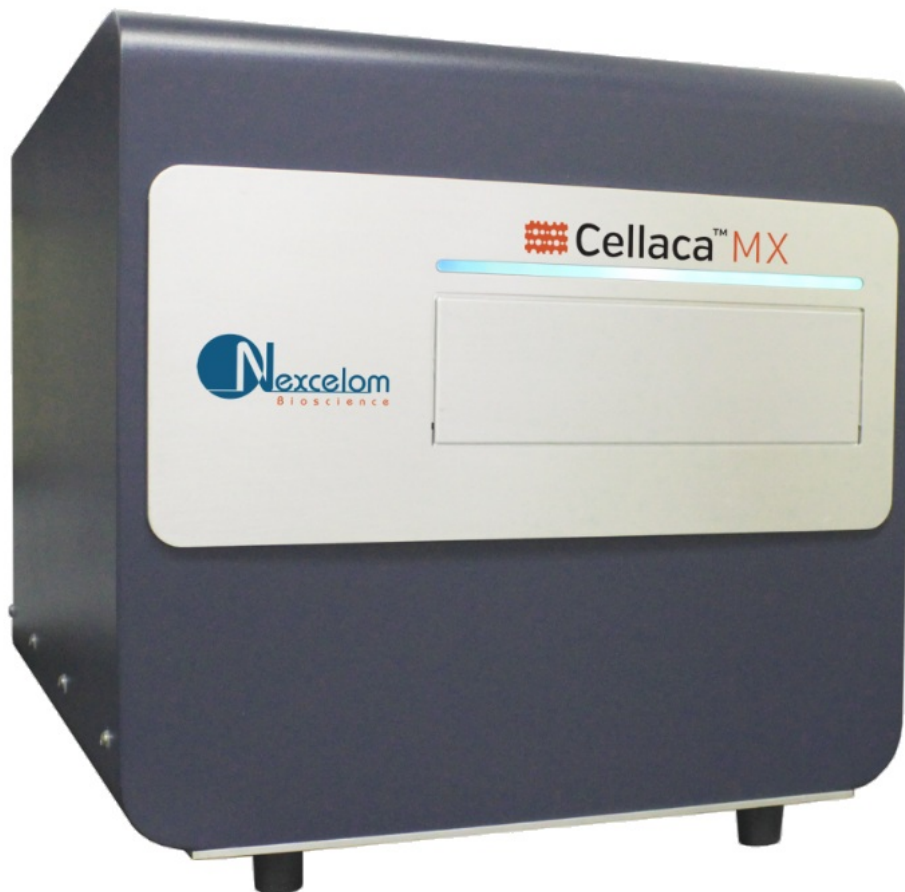
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Cellaca[®]

Cellaca MX High Throughput Automated Cell Counter



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 12×2 and 8×3 plate layouts
- Focus Guide – Graphic sheet to help with adjusting instrument focus
- Nexcelom Counting Plates (24-well, 12×2 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.

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.



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 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.

Staining Solution Guidelines

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

<i>Stain Type</i>	<i>Use with Cell Sample</i>	<i>Dilution Factor</i>
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.
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.

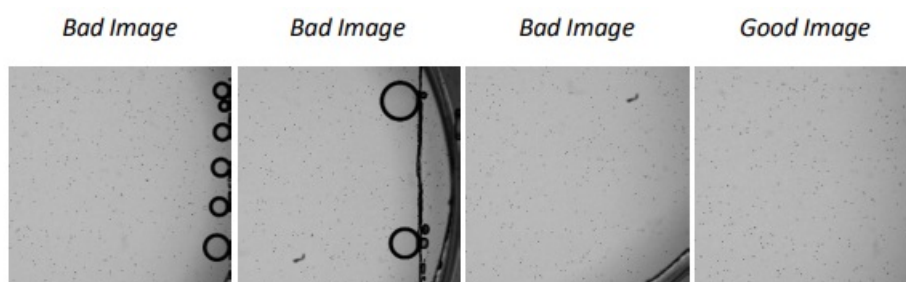
To select or clear multiple wells, click a well and hold/drag your mouse to encompass other wells. To select or clear all wells, click the button.

9. Click the Preview button to view the sample.

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

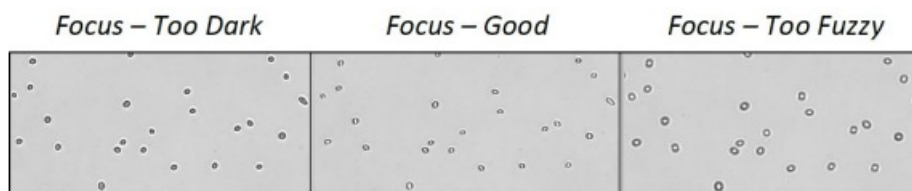
Performing Image Clipping Check

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.

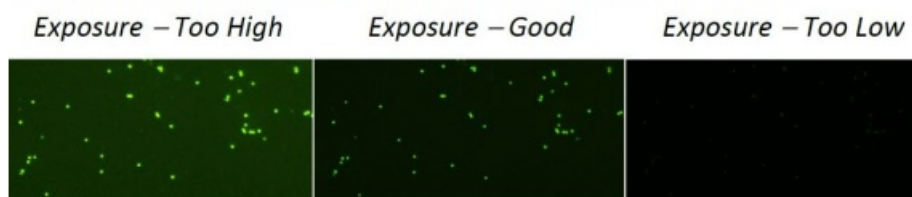


Understanding Instrument Focus

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

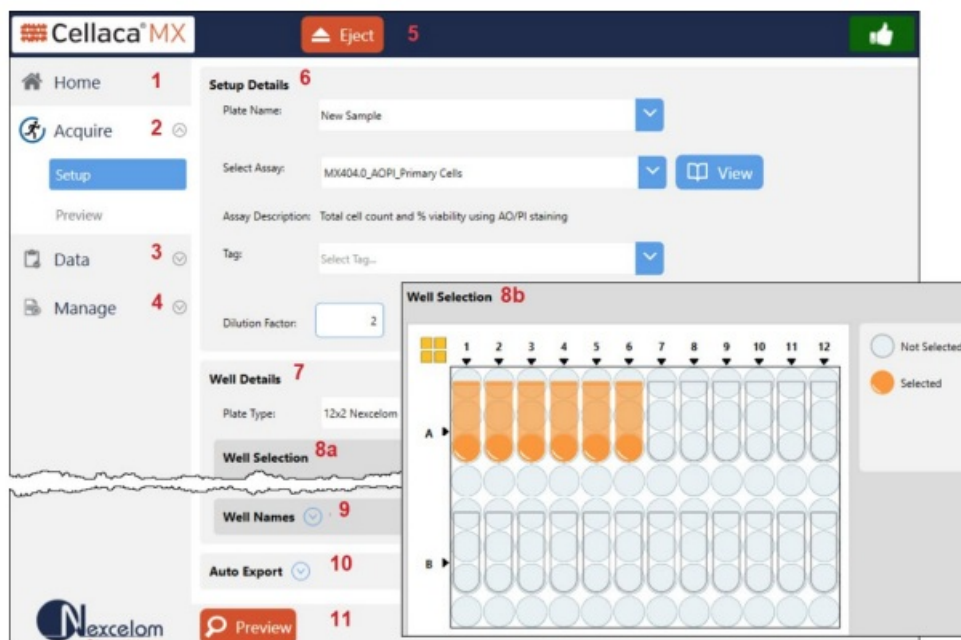


Fluorescent signals should be strong with a low, dark background.



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.

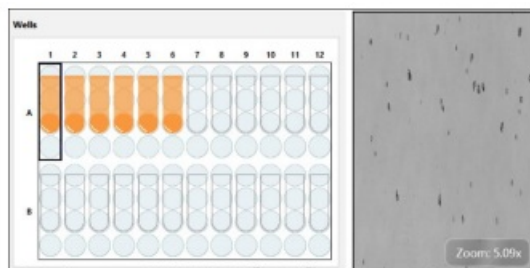
Entering Setup Details

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., 12×2 or 8×3 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.



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.



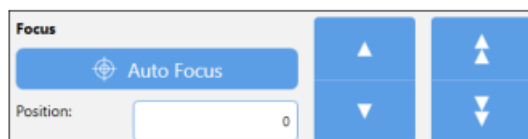
3. To view other available channels (i.e., channel display is based on selected assay), click the FL buttons accordingly. For example, sample Channel 1|FL and Channel 2|FL images are shown below.



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**

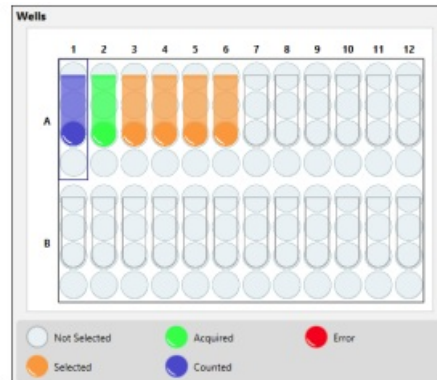
Allows users to finely adjust vertical (Z) position of the objective lens for optimal focus (in μm). Click the up/down single arrows to adjust focus.

- **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.

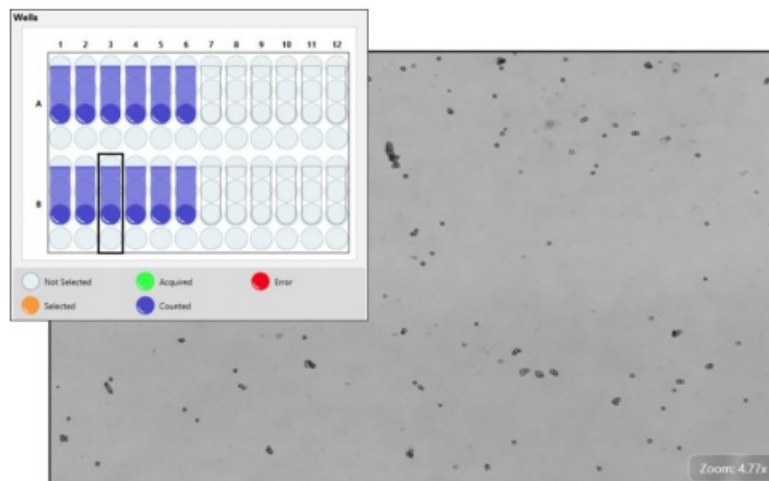
5. Click the Count button.

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.

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.

- 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).
- 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	

- 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 View Summary .csv				
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.

- Confirm the current export location. Click Browse, navigate to a folder on your Operating Computer or network and click OK.
- 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.
- 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.
- 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).

Plate Name	Plate Type	Assay	Imaging Mode	Tag	Scan Creation	Result Creation	Magnification	Product Type
New Sample 2021/11/19-13:54:29	12x6 Nucleom	M0404_2_AOP1	2 Brightfield and 2 Fluor	AOP1	11/19/21 13:54:32	11/19/21 13:54:32	2.4	Mx
New Sample 2021/11/19-13:51:12	12x6 Nucleom	M0404_2_AOP1	2 Brightfield and 2 Fluor		11/19/21 13:51:16	11/19/21 13:51:16	2.4	Mx
New Sample 2021/11/18-12:48:37	CH14-e0100	K2_v4_AOP1_Pri	2 Brightfield and 2 Fluor		11/18/21 12:51:31	11/18/21 12:51:31	4.0	K2

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 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.

Name	Description	Product	Imaging Mode	Created	Modified	State	Shown
MX403.1_A375_GFP_Transfection Rate	Total Cell Count and % GFP	Mx	BR/FL	6/25/2020 3:49:14 PM	9/21/2021 8:25:04 AM	🔒	✓
MX404.1_ACP1_Splenocytes	Total cell count and % viabi	Mx	2 BR/FL	6/25/2020 3:52:12 PM	9/21/2021 10:40:38 AM	🔒	✓
MX_u4_Dry Demo ROS		Mx	BR/FL	8/28/2020 9:54:05 AM	9/21/2021 10:48:48 AM	🔒	✓
MX_u4_Dry Demo Caspase 3		Mx	BR/FL	9/9/2020 10:14:49 AM	9/21/2021 10:51:50 AM	🔒	✓
MX_u4_Dry Demo Calcein AM-PI		Mx	2 BR/FL	8/28/2020 9:54:34 AM	9/21/2021 10:54:59 AM	🔒	✓
MX_u4_Dry Demo Annexin V PI		Mx	2 BR/FL	8/28/2020 10:48:19 AM	9/21/2021 11:03:00 AM	🔒	✓
MX401.0_Typing Blue Viability	Total Cell Count and % viabi	Mx	BR	9/26/2019 9:06:00 AM	9/21/2021 11:22:02 AM	🔒	✓

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 1.0×10^5 – 1.0×10^7 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 μ L of cell sample with 50 μ L 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, 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 1.0×10^5 – 1.0×10^7 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 μ L of cell sample with 50 μ L 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 μ L	50 μ L	20
PBMCs following Ficoll separation	<i>Not Required</i>	50 μ L	50 μ L	2
Mononuclear cells from processed bone marrow	<i>Not Required</i>	50 μ L	50 μ L	2
Tumor digest/ Tissue digest	<i>Not Required</i>	50 μ L	50 μ L	2
Stem cells from CD34+ separation	<i>Not Required</i>	50 μ L	50 μ L	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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Documents / Resources

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Manuals+