

Contents of Shipping Container

- Cellometer K2 Instrument**
- Instrument **Power Supply** and **Power Cord**
- USB 2.0 Connector Cable**
- USB Drive** – Contains **Matrix** software and PDF files listed below
- Cellometer K2 Matrix User Manual** (PDF file on USB Drive/Laptop)
- Matrix Software User Manual** (PDF file on USB Drive/Laptop)
- Cellometer K2 Matrix Quick Start Guide** (PDF file on USB Drive/Laptop – *this document*)
- Cellometer K2 Focus Guide** – Graphic sheet to help with adjusting instrument focus
- Revvity Counting Chamber Slides** – One box of 75 disposable slides
- AO/PI Viability Reagent** and **Starter Kit** (FL Beads and two SD100 slides)
- Revvity-provided Laptop** with **Power Supply** and **Power Cord** (if purchased with instrument; shipped separately)

Unboxing the Instrument

Unpack and visually inspect the K2 to ensure no physical damage has occurred during shipping. For assistance in setting up the instrument, visit the Cellometer K2 page on our website for training videos on unboxing and getting started.

As the K2 instrument can be run using either the *Cellometer Image Cytometer* software or the *Matrix* software, the logo distinguishes the K2 *Matrix System* from earlier versions.



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 Cellometer K2.

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 *K2 Matrix 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 scan results.

If you purchased a Revvity-provided Laptop...

If you purchased a Revvity-provided Laptop with the K2 instrument, Matrix software is pre-installed and you can skip installation as described in this guide. Continue to *Using Instrument for the First Time* on page 3.

If you supplied your own Operating Computer...

A customer-supplied Operating Computer set up to to run the K2 powered by Matrix software must meet the following *minimum* requirements:

- Windows 10 PC, 64-bit
- Intel® Core i7, 2.0 GHz Processor
- 16 GB RAM
- USB 2.0
- GPU NVIDIA P1000 (or equivalent)
- Full HD Display Resolution: 1920 x 1080 pixels

Installing K2 Matrix System Software

If you purchased a Revvity-provided Laptop with the K2 instrument, Matrix software is pre-installed and you can skip this section. Continue to *Using Instrument for the First Time* on page 3.

K2 Matrix System software installation consists of multiple steps including *Preparing for Installation*, *Installing the Matrix Software*, *Restoring the Matrix Database* and *Entering Instrument Details*. Once installation is complete, additional steps are required when using instrument for the first time such as *Connecting Instrument to Operating Computer*, *Launching the Matrix Software*, *Verifying Instrument and Camera Connections* and *Calibrating Instrument via the Software*.

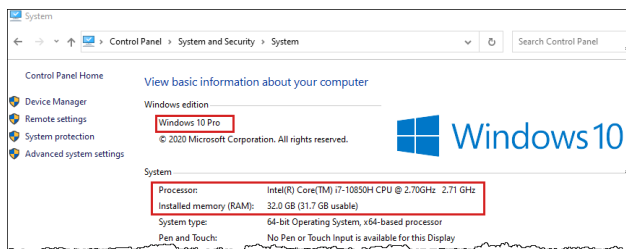
Note: Ensure that instrument is *not* connected to the Operating Computer during installation until specified in this procedure.

Preparing for Installation

Preparing for installation includes verifying that a customer-supplied Operating Computer meets Matrix software *minimum* requirements.

Verifying Minimum Operating Computer Requirements

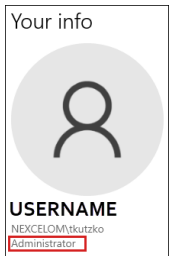
1. Navigate to the *Control Panel*.
2. Click *System and Security*, then *System* to view basic information about the computer.



3. Confirm Operating Computer meets or exceeds Matrix software minimum requirements as specified on page 1.
4. Close the window by clicking **X** in the upper right corner of screen.

Confirming Installation Permissions

1. Navigate to the *Control Panel*.
2. Click *User Accounts* and then *User Accounts* again.
3. Click *Make Changes to my account in PC settings*.
4. Ensure that the *Administrator* privilege is displayed below your account information.



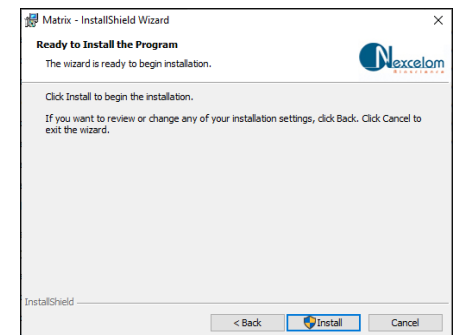
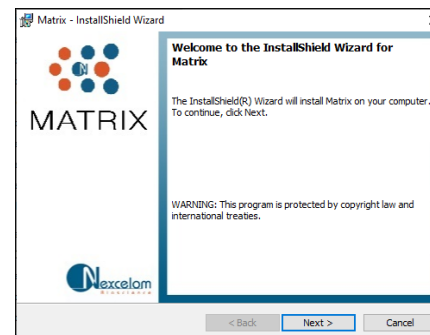
Installing the Matrix Software

If the Matrix 21 CFR Part 11 module is to be enabled, you must contact Support *after* software installation is complete for assistance.

1. Insert USB Drive containing Matrix software into USB port.
2. Navigate to USB drive and copy folders/files to indicated locations:
 - Copy **K2 Settings** folder and **Matrix_vN.N_N.N.NNNNN.N** folder (where *N.N* is version and *NNNNN.N* is build number) to desktop.
 - Copy **Matrix_vN.N_N.N.NNNNN.N.bak** file to root folder (e.g., **C:**).
3. Open **Matrix_vN.N_N.N.NNNNN.N** folder on desktop and in **DISK1** subfolder, double-click the **MatrixSetup.exe** installer file.

Note: The installer checks for prerequisites such as C++ and MS SQL Server on your system. If prerequisites need to be installed, you may need to restart your computer and run the **MatrixSetup.exe** file again.

4. When the *Welcome* screen for the installer is displayed, click **Next**.

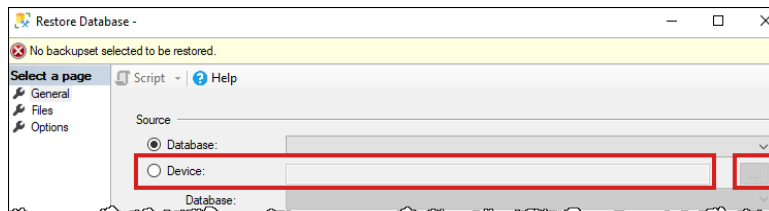
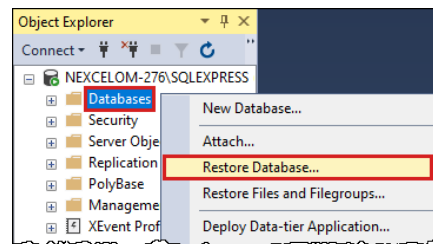
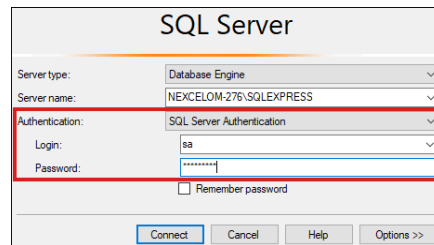


5. Click **Install**.
6. Click **Finish**.

7. Confirm that the **Matrix** icon appears on desktop.
8. Open the **K2 Settings** folder on desktop and then copy the **Configurations** folder contained within to the following path:
`<root_folder>:/ProgramData/Revvity/Matrix/vNNN`
 where *NNN* represents version number (e.g., *510* is version *5.1.0*).
9. When prompted with a confirmation message, click the **Replace files in destination** button and close the window.

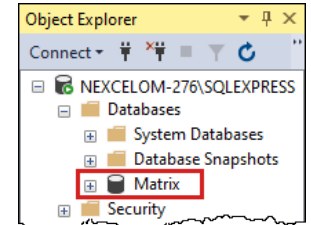
Restoring the Matrix Database

1. From the *Windows Start Menu*, expand the **Microsoft SQL Server Tools 18** folder to launch **Microsoft SQL Server Management Studio 18**.
2. In the *Authentication* drop-down, select **SQL Server Authentication**.
3. Log in using the following credentials – Login: **sa**
Password: **Cellaca01**
4. Click **Connect**.
5. On the left side of the screen, right click on **Databases** and select **Restore Database** from the menu.
6. In the *Source* area, select **Device** and click the ... button.



7. Click the **Add** button.
8. Navigate to the location in your root folder (e.g., **C:**) where you copied the **Matrix_vN.N_N.N.NNNNN.N.bak** file. Select the file and click **OK**.

9. When prompted with a confirmation message, click **OK** to confirm the file and then click **OK** to restore the database.
10. Expand the database tree and verify that the **Matrix** database appears in the list.
11. Close the window by clicking **X** in the upper right corner of the screen.



K2 Matrix System software installation is now complete.

Enabling the 21 CFR Part 11 Module

If the *Matrix 21 CFR Part 11* module is to be enabled for your system (i.e., requires purchase of a module license), you must contact Support *at this point* for assistance.

Using Instrument for the First Time

If you are accessing the Matrix software from a touchscreen device, you can interact directly with the 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 touchscreen 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.

Set up Operating Computer (Laptop) within reach of the K2 by connecting its Power Supply/Power Cord, plugging it into an electrical outlet, powering it on and waiting for the software to initialize. *The USB 2.0 Connector Cable must reach the computer while it is connected to the instrument.*

Connecting Instrument to Operating Computer

1. Connect instrument Power Supply to Power Cord.
2. Connect instrument Power Supply/Power Cord to the K2.
3. Plug instrument Power Cord into a surge protector (*recommended*) or an electrical outlet.
4. Turn instrument Power Switch (located on back left) to *ON* position. *Light on front of unit will be lit.*
5. Connect USB Cable to the K2.

- Connect USB Cable to USB 2.0 port on Operating Computer. *Be sure that software installation is complete before connecting USB Cable. USB driver will be downloaded automatically by the Operating Computer.*

Launching the Matrix Software

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





Note: Upon launch of the software the instrument runs through a startup sequence that includes connecting to the database and initializing the calibrations.

If using a customer-supplied Operating Computer, you will also be prompted to enter instrument details.

Entering Instrument Details

- Enter *Serial Number* (located on back of unit).
- Enter *Optics Number*.
- Enter *Filter 1* (i.e., *Optics Module A #*).
- Enter *Filter 2* (i.e., *Optics Module B #*).
- Click **OK**.

Verifying Instrument and Camera Connections

- Click the  icon located in upper right corner of the screen.
- Confirm that text in the dialog box displayed indicates instrument and camera are connected to the Matrix software.
- Click the  icon again to close the dialog box.



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 chamber slide loaded in the instrument. Contact 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.

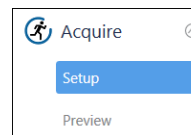
The *Header Bar* visible across the top of the screen contains instrument status icons. It also displays current assay and plate name as you move through the data acquisition and analysis workflows.



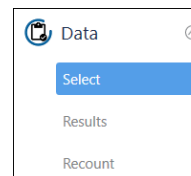
The *Navigation Bar* visible on the left side of the screen is always accessible. Functionality associated with each tab is described below. See the *Matrix Software User Manual* for full details on using tab functions.



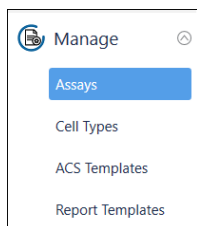
Home Tab: Displays the Powered By  logo distinguishing the software from prior versions. Also contains the **About Matrix** button which displays version details and Revvity contact information.



Acquire Tab: Contains the two sequential screens in the *Data Acquisition* workflow. For example, you must first enter *Setup* details before advancing on to *Preview* the sample and confirm focus/fluorescent exposure.



Data Tab: Contains the three sequential screens in the *Data Analysis* workflow. For example, you must first *Select* a scan result before you can view 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 *Assays*, *Cell Types*, *ACS Templates* and *Report Templates* that can be 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 assays, cell types and report templates.

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

See *Evaluating Viability Methods* on page 9 for details on how to choose a staining method based on the cell sample and selected assay type.

Preparing Slides with Samples

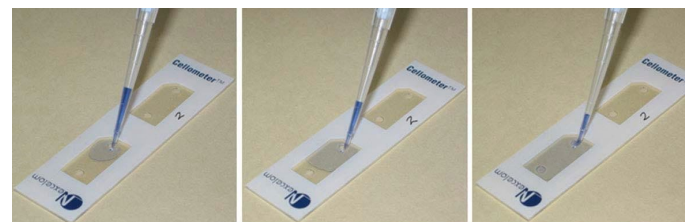
Although the K2 does not require routine testing or calibration, counting beads are available to verify instrument functionality. *Revvity counting beads CCBM-011-2ML are recommended for use with K2.*

To prepare sample slides with counting beads or cell samples:

- For SD100 slides, remove protective film from both sides of the slide. *PD100 slides do not have this film.* Note that it may be difficult to peel film from the slide.
- Place the slide on a fresh Kimwipe.
- To prepare two samples at once, label individual chambers (e.g., # 1 and # 2) in the white area. Take care to ensure that the clear optical windows of the counting chamber are *not* touched.



- Invert counting bead solution or tube containing cell sample a total of 10 times (10x). *If using beads, vortex solution for 10 seconds. Do NOT vortex cell samples.*
- Set pipette to 20 μ L and then pipette bead solution/ cell sample up and down ten times (10x) to break up potential clumps.
- Load 20 μ L of bead solution/cell sample into induction port of a slide counting chamber (or “well”). If needed, repeat process to load second chamber of slide. *Liquid will move into imaging area for each filled well.*



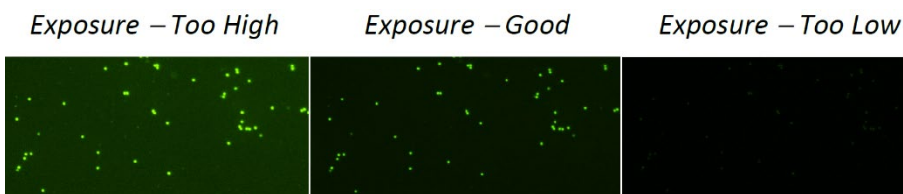
- Hold the loaded chamber in white area and insert counting chamber containing sample into Sample Slot until touching the internal stop.

Understanding Instrument Focus

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

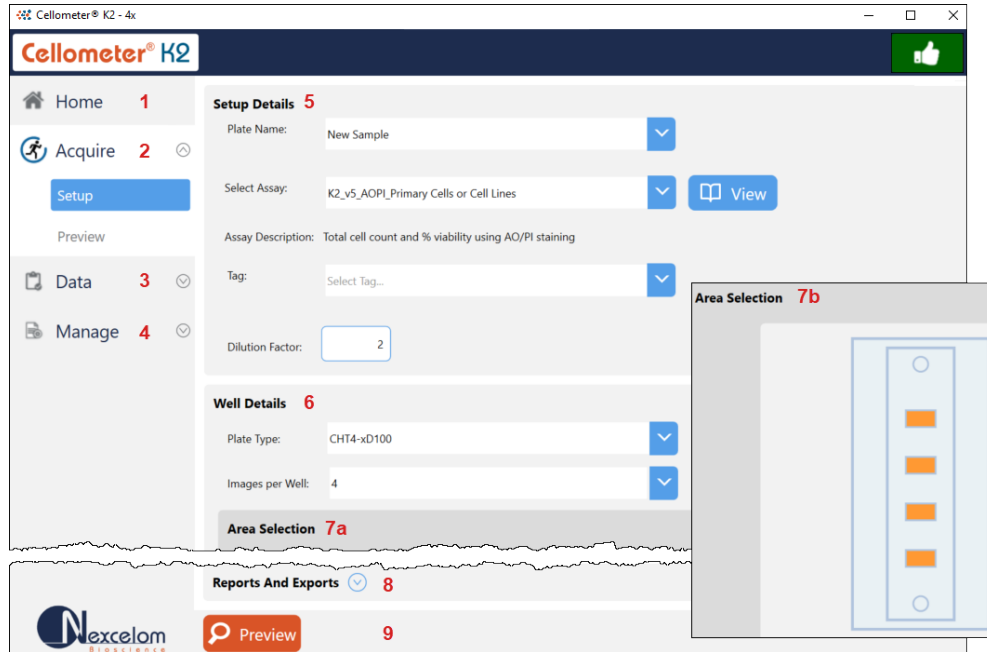


Fluorescent signals should be strong with a low, dark background.



Counting and Analysis Workflow

Performing a cell count consists of entering *Setup Details* and previewing the live image before viewing scan 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 ACS/report template lists.
- 5 Setup Details Area** – Use to enter plate name, select an assay, add a tag (to group scan results for custom reporting) and dilution factor.
- 6 Well Details Area** – Use to enter slide type and number of *Images Per Well*.
- 7 Area Selection** – Displays slide visualization of *Images Per Well* selected. *Users can select areas in this visualization when previewing live images.*
- 8 Reports and Exports Area** – Use to specify auto export location.
- 9 Preview Button** – If slide is loaded and *Setup* details have been entered, displays live images to adjust focus/fluorescence exposure.

Entering Setup Details

- In the **Acquire** tab, *Setup* screen, enter a **Plate Name**. *If you do not enter a plate name, a date/time stamp is appended to the “New Sample” default (e.g., New Sample 2023/08/25-10:58:09).*
- 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.
- Select **Plate Type** and **Images Per Well** to be captured during the scan. *The slide visualization depicts selected number of images (i.e., 1, 4 or 8). Each image represents a cross-section of the area in the counting chamber or “well”.*
- If desired, expand the *Reports and Exports* area to modify the default auto export location.
- Click the **Preview** button.

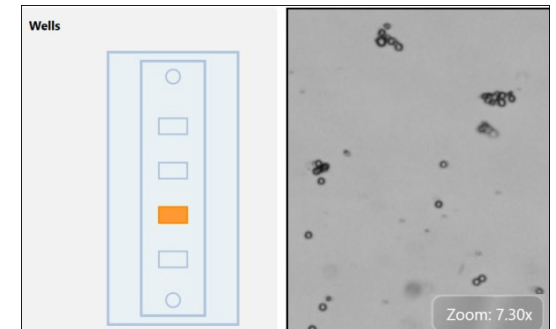
Previewing Live Images

The Preview screen displays live images for areas selected in the well.

- In the *Wells* area, click on individual areas (i.e., cross-sections of the counting chamber or well). As you move from area to area, the live image displayed will change per the selected image.

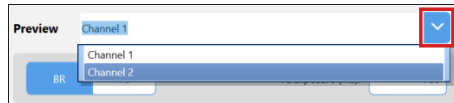
To zoom in and out of an image, move the mouse to hover cursor over

the viewing pane and turn the scroll wheel or, if using the touchscreen, apply universal gestures (e.g., touch the center of the image with two fingers and then slowly spread them apart to zoom in and reverse this action to zoom out.) *Zoom* magnification is displayed in the bottom right corner of the viewing pane.

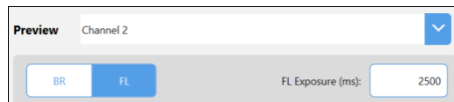


To move a zoomed image around, click and drag to a new location.

- Adjust focus for *BR* image by slowly turning the instrument Focus Knob.
- In the *Preview* area, click the drop-down to view available channels. *Channels displayed will depend on assay Imaging Mode.* Select a channel to change image display.



- 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.



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 two channels, use the *Channel 1/BR* image to adjust focus and then select the *FL* image to confirm exposure. For *Channel 2*, you only need to select the *FL* image to confirm exposure since focus of its paired *BR* image is adjusted automatically. In addition, a slight offset may exist between *BR1* and *BR2* images due to the distance the camera must travel.

- Click the **Count** button.

Analyzing Scan Results

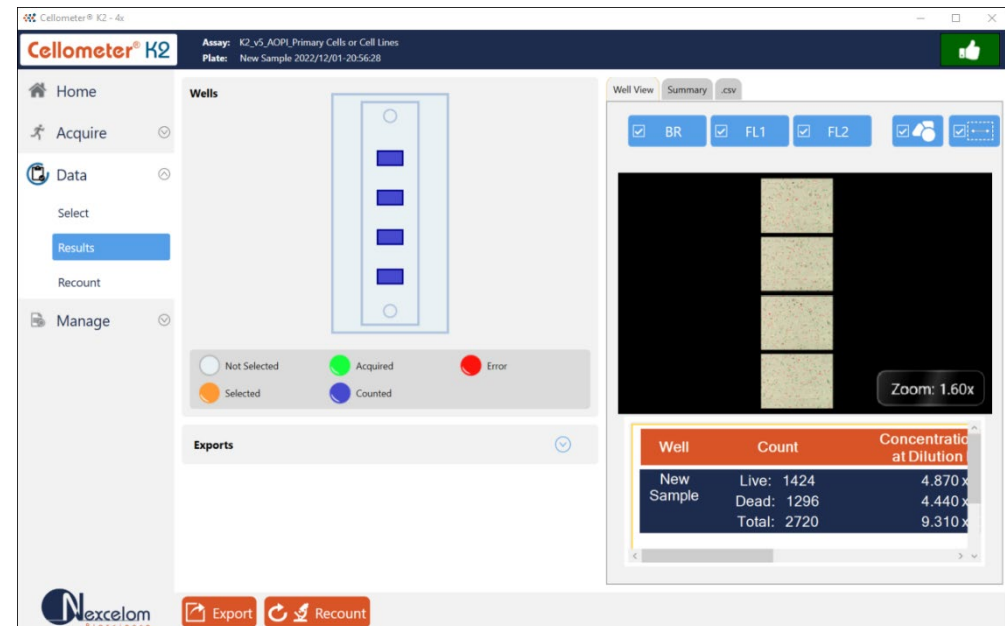
As the system acquires sample images, the colors used to mark well areas (where the number of highlighted areas is based on *Images Per Well* value) change to indicate image status (i.e., from *Selected* to *Acquired* to *Counted*) as shown in the legend displayed below the slide visualization.



Area images are displayed in the **Well View** tab along with acquired data for the sample 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, a single image is displayed in the viewing pane for the sample well. If an **Images Per Well** value of 4 or 8 was selected, click on the composite image (containing either 4 or 8 stacked images of areas in the well as presented in the slide visualization) and drag to view all areas.



- At the top of the **Well View** tab, click the *Brightfield (BR)* or *Fluorescence* (e.g., **FL1** and **FL2**) buttons to select/de-select channels used in display of the image. *Channel views are overlaid on top of each other.*
- Click the 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 specified cell diameter).*
- Click the 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.*

- At the bottom of the **Well View** tab is a report containing well-level details for the sample image. *If an **Images Per Well** value of 4 or 8 was selected, the report represents consolidated data from all images.*

Well	Count	Concentration (cells/mL) at Dilution Factor: 2	Mean Size (um)	Viability: 52.0 %
New Sample	Live: 1424	4.870x10 ⁶	9.1	
	Dead: 1296	4.440x10 ⁶	9.3	
	Total: 2720	9.310x10 ⁶	9.2	

- Click the **Summary** tab to view a full-page containing count results.

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.

Recount Details

Last Used Assay: K2_v5_AOPI_Primary Cells or Cell Lines View

Assay for Recount: K2_v5_AOPI_Primary Cells or Cell Lines View

Assay Description: Total cell count and % viability using AO/PI staining

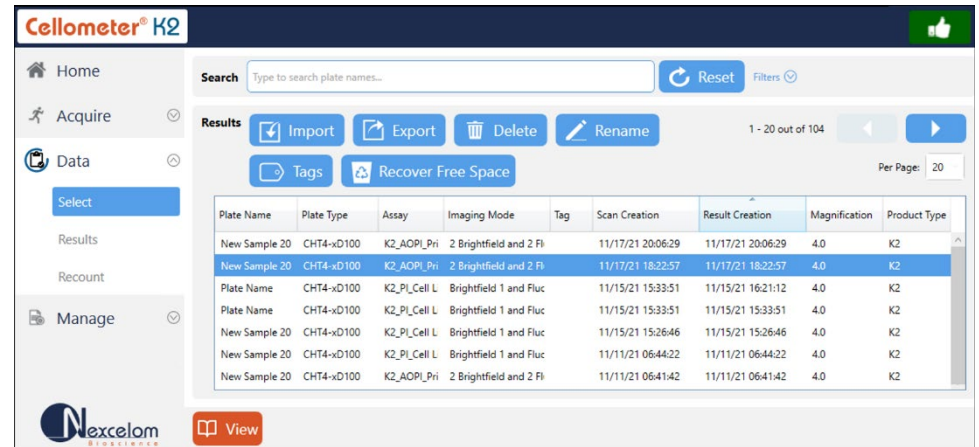
Tag: Select Tag... View

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** 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 to view its data before performing a recount where you can fine-tune assay/cell type parameter settings).



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, analyze data by clicking channels and varying the presentation of images. 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.*

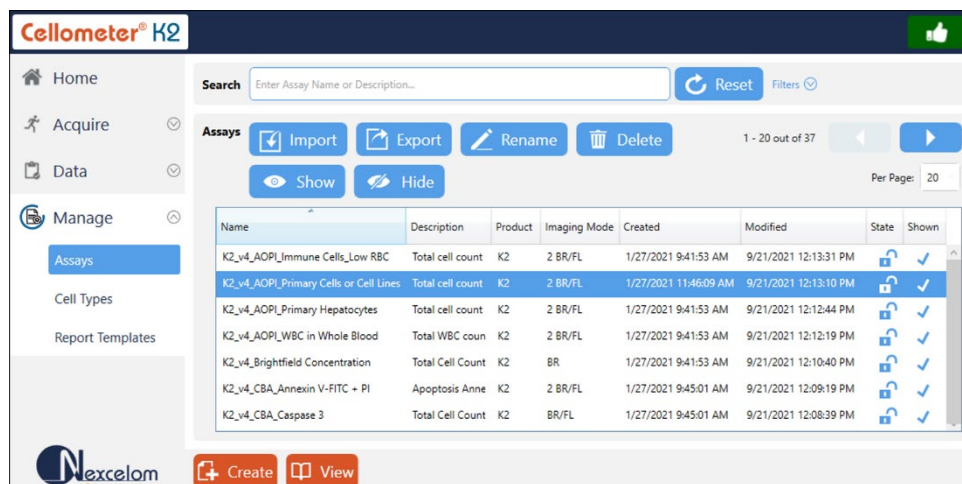
To fine-tune assay parameters for the scan result, you can click the **View** button for either the *Last Used* assay or a selected new assay and edit its parameters as necessary. To perform a recount, click the **Recount** button.

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 *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.



The screenshot shows the Cellometer K2 software interface. The 'Manage' tab is active, displaying a list of assays. The interface includes a search bar, a 'Reset' button, and a 'Filters' icon. Below the search bar are buttons for 'Import', 'Export', 'Rename', and 'Delete'. There are also 'Show' and 'Hide' buttons. The table below shows a list of assays with columns for Name, Description, Product, Imaging Mode, Created, Modified, State, and Shown. The 'State' column contains icons for lock and unlock, and the 'Shown' column contains checkmarks.

Name	Description	Product	Imaging Mode	Created	Modified	State	Shown
K2_v4_AOPI_Immune Cells_Low RBC	Total cell count	K2	2 BR/FL	1/27/2021 9:41:53 AM	9/21/2021 12:13:31 PM	🔒	✓
K2_v4_AOPI_Primary Cells or Cell Lines	Total cell count	K2	2 BR/FL	1/27/2021 11:46:09 AM	9/21/2021 12:13:10 PM	🔒	✓
K2_v4_AOPI_Primary Hepatocytes	Total cell count	K2	2 BR/FL	1/27/2021 9:41:53 AM	9/21/2021 12:12:44 PM	🔒	✓
K2_v4_AOPI_WBC in Whole Blood	Total WBC coun	K2	2 BR/FL	1/27/2021 9:41:53 AM	9/21/2021 12:12:19 PM	🔒	✓
K2_v4_Brightfield Concentration	Total Cell Count	K2	BR	1/27/2021 9:41:53 AM	9/21/2021 12:10:40 PM	🔒	✓
K2_v4_CBA_Annexin V-FITC + PI	Apoptosis Anne	K2	2 BR/FL	1/27/2021 9:45:01 AM	9/21/2021 12:09:19 PM	🔒	✓
K2_v4_CBA_Caspase 3	Total Cell Count	K2	BR/FL	1/27/2021 9:45:01 AM	9/21/2021 12:08:39 PM	🔒	✓

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 1.0×10^5 – 1.0×10^7 cells/mL can be analyzed on the K2, 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 20 μ L of cell sample with 20 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 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* (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 20 μ L of cell sample with 20 μ L 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.

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

Available Product Documentation

See the following documentation for additional instrument information:

- **8003393 Cellometer K2 Matrix User Manual** for instrument operation, care and maintenance details (available as a PDF on either the USB Drive or desktop of Revvity-provided Laptop shipped with instrument).
- **8003394 Matrix Software User Manual** for complete details on using software functionality (available as a PDF on either the USB Drive or desktop of Revvity-provided Laptop shipped with instrument).
- **8001583 Cellometer K2 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.