Cellometer™ Ascend Quick Start Guide

8004709 Rev B

Contents of Shipping Container

- ☐ **Cellometer Ascend Instrument**
- ☐ **Power Supply** and **Power Cord**
- ☐ **Matrix Software** (pre-installed on instrument)
- ☐ **USB Drive** containing user documentation listed below:
  - *8004707 Cellometer Matrix Software User Manual*
  - *8004708 Cellometer Ascend User Manual*
  - *8004709 Cellometer Ascend Quick Start Guide (this document)*
  - *8004710 Cellometer Ascend Focus Guide*
- ☐ Box of 100 **3-Chamber Slides** (ASD-CHM3-001)
- ☐ Five **8-Chamber Slides** (not for individual sale; ASD-CHM8-001 for a box of 100 slides)
- ☐ One 0.5 mL vial of **Fluorescent Counting Beads** (not for individual sale)
- ☐ One 1.0 mL vial of **AO/PI Viability Reagent** (not for individual sale; CS2-0106-5mL for a 5 mL vial)

Unpacking and Setting Up Instrument

Unpack and visually inspect the Ascend to ensure no physical damage has occurred during shipping.

1. Connect Power Supply to Power Cord.
2. Connect Power Supply to instrument (via input on back panel).
3. Plug Power Cord into a surge protector (recommended) or an electrical outlet.

The Ascend is an all-in-one standalone instrument offering an integrated touchscreen. Ports are available on the front and back for linking to a network, accessing external files, connecting to printers, and storing count scan results.

Site Preparation

Instrument must be placed on a level surface and plugged directly into an electrical outlet. *Use of a surge protector is recommended.*

Follow all equipment safety protocols, and keep the area around instrument clean both during and post operation.

Launching the Matrix Software

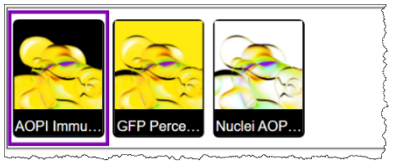
As the Ascend is a touch-screen device, tap gently on Matrix screen elements (e.g., tabs, icons, dropdowns, or buttons) using a finger or stylus pen to make software graphical user interface (GUI) selections. As an alternative, a USB mouse and keyboard may be connected.

1. Turn instrument Power Switch (located on lower left side of back panel when facing instrument) to *ON* position.
2. To power on instrument, press the Power Button (located on front panel below Sample Slot). *Light Bar above sample slot will be lit and internal Operating Computer will run through startup sequence.*
3. On the tablet touchscreen, double-tap the Matrix icon to launch the software.

Simplified Workflow

Simplified workflows have been integrated into the Matrix software on the Ascend to streamline user input during data acquisition and analysis.

The *Favorite selection* panel is displayed across the top of the Setup screen and highlights either the last used favorite or the system default. To run the selected favorite, simply insert the slide.



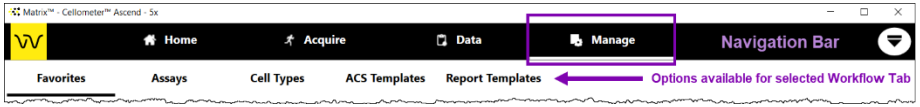
Favorites enable users to instantly run pre-set counting parameter settings. Cell assays that are performed frequently can be run with minimal software interaction.

Matrix Screen Elements

Upon launching the Matrix software, you are presented with the **Acquire** workflow tab Setup screen by default. 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 the top of the screen is always displayed and contains workflow tabs.



Functionality associated with each tab is described below.

Home Tab: Displays the instrument and Matrix software logos. The **About Matrix™** button 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).

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 re-analysis creating 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.

Preparing Slides

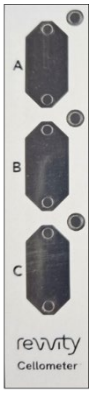
Although the Ascend does not require routine testing or calibration, counting beads are available to verify instrument functionality. *Revvity counting beads CCBM-011-2mL are recommended for use with Ascend.*

To prepare slides with counting beads or cell samples:

1. For *3-Chamber* and *8-Chamber* slides, remove protective film from both sides of the slide. Do *not* touch or write on the clear optical window areas of the slide.

   Note: It may be difficult to peel protective film from the slide. One method is to adhere tape to the top of film and then pull tape off (with film attached).

2. Place slide on a fresh Kimwipe.
3. To prepare multiple samples on the same slide, label individual counting chambers (e.g., *A, B, C, etc.)* in the white margin. Be careful to *not* touch the clear optical window of the counting chamber. *Ascend 3-Chamber slides have already been labeled with A, B, and C.*



Loading Slides with Samples

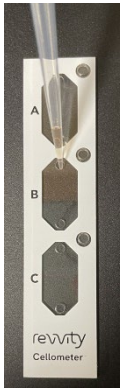
Cellometer Ascend *3-Chamber* and *8-Chamber* slides consist of independent chambers manufactured to a precisely controlled height allowing for cell concentration calculation.

1. To prepare cells for counting, invert tube containing sample 10 times, then pipette up and down 10 times. This will help to evenly suspend cells. Do *not* shake or vortex sample as it may damage cell membranes.

   Note: If measuring cell viability (*optional*), stain sample using a 1:1 ratio of chosen viability stain (e.g., 0.2% trypan blue, AOPI, or other) and mix by pipetting up/down 10 times *before* performing *Step 1*, above.

   When using trypan blue for cell viability, ensure that stock concentration used is 0.2%. If stock concentration is 0.4%, it is recommended to dilute it with a balanced salt buffer (PBS) followed by a 0.2 µm filtration step before staining sample.

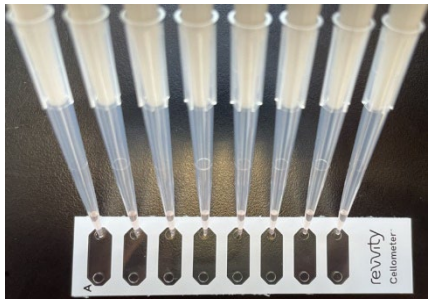
2. Immediately load the recommended volume of cell suspension for each counting chamber (noted below) by pipetting directly into the inlet port.



   – For *3-Chamber* slides, load 20 µL per chamber.
   – For *8-Chamber* slides, load 10 µL per chamber.

   Touch pipette tip to a chamber's inlet port and slowly pipette entire recommended sample volume per chamber all at once. Capillary force automatically spreads the sample within the chamber. Each counting chamber can hold a separate sample as there is no mixing between individual chambers on a slide.

   Ascend 8-Chamber slides are compatible for use with a standard multi-channel pipette.



Streamlined User Experience

Ascend is unique in the Cellometer family of instruments in that it requires minimal user interaction with the software.

- To initiate the counting and analysis workflow for the Ascend, users can simply select a favorite from the Matrix software and insert a loaded slide into the Sample Slot.
- The instrument automatically retracts the slide and, if the *Skip Preview* feature is enabled in the selected favorite, begins the counting process.
- When processing is complete, the slide is automatically ejected from the instrument and data results are displayed/exported for analysis.

Ascend Auto Focus Methods

The Ascend offers the following two auto focus methods.

Image-Based Auto Focus

The image-based auto focusing algorithm performs a *Z-stack* (i.e., compilation of images at different focal planes) of the sample in the slide chamber. While collecting these images from the Z-stack, the software analyzes objects in each image and selects the focal plane with best contrast for sample acquisition. Users have the option to perform image-based auto focusing for either the brightfield or fluorescent channels.

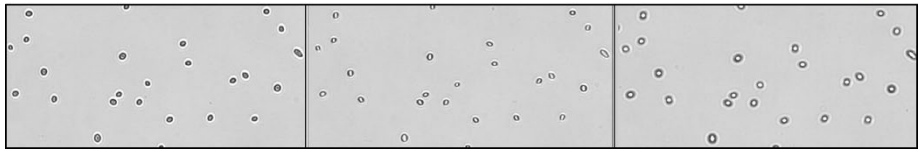
Slide-based Auto Focus

Available only for the 3-Chamber Slide on the Cellometer Ascend. The Slide-based auto focusing operation allows the software/instrument to focus on the consumable (without needing to focus directly on the sample) by using a proprietary slide technology.

Understanding Instrument Focus

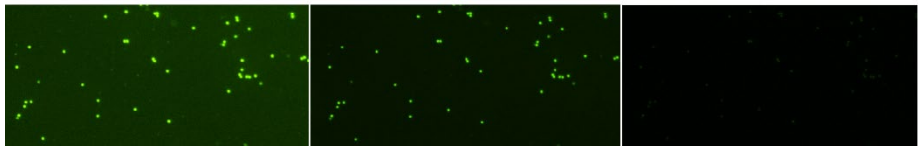
Live cells should have a bright center and dark, crisp clearly defined edges.

Focus – Too Dark *Focus – Good* *Focus – Too Fuzzy*



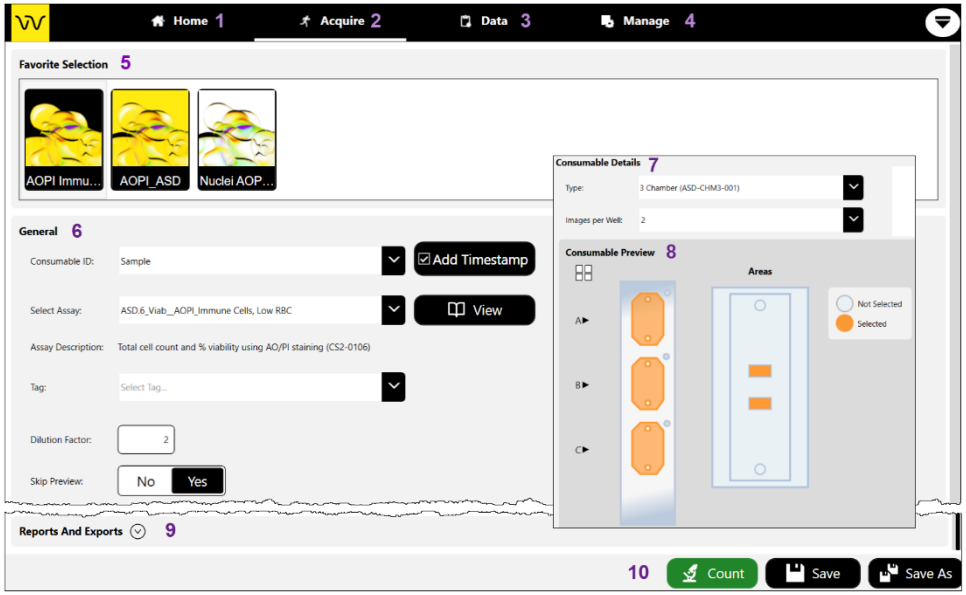
Fluorescent signals should be strong with a low, dark background.

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



Counting and Analysis Workflow

The *Counting and Analysis* workflow consists of choosing image parameter settings, previewing the live image *(if **Skip Preview** field is set to **No**)* to adjust instrument focus and exposures, and performing a count.



1. **Home Tab** – Displays Home screen with instrument/software details.
2. **Acquire Tab** – Use to select favorites and enter scan parameter settings.
3. **Data Tab** – Use to view current scans/select saved scans for re-analysis.
4. **Manage Tab** – Displays *Favorites*, *Assays*, *Cell Types*, *ACS Templates*, and *Report Templates* options for managing system lists.
5. **Favorite Selection Area** – Displays favorites available for quick use.
6. **General Area** – Use to enter consumable ID, select an assay, add a tag, specify a dilution factor, and choose whether to skip Preview screen.
7. **Consumable Details Area** – Use to enter slide type and *Images Per Well*.
8. **Consumable Preview Area** – Displays visualization of consumable *Type* and *Images Per Well*. *Users can select wells and areas during preview.*
9. **Reports and Exports Area** – Use to specify auto export location.
10. **Preview** or **Count Button** – Tap **Preview** (if **Skip Preview** is set to *No*) to view live images or **Count** (if **Skip Preview** is *Yes*) to perform a count.

Choosing Data Acquisition Method

1. In the Navigation Bar, tap the **Acquire** tab (if not already displayed by default) to display the Setup screen.
2. Either select a favorite from the *Favorite Selection* panel or choose an assay and customize parameter settings for a count.

Selecting a Favorite

Scroll across the *Favorite Selection* panel and tap to select a favorite. To run the selected favorite, insert the slide.

If **Skip Preview** field is set to *Yes* in the favorite's parameter settings, the inserted slide will automatically be counted. If *Skip Preview* feature is set to *No*, users can preview samples and adjust exposures (see *Preview Mode* on page *5*) prior to acquisition and counting (see *Count Mode* on page *5)*.

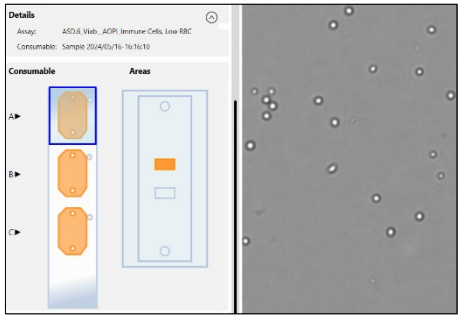
Selecting an Assay

1. In the *General* area, enter a **Consumable ID**. *If a Consumable ID is not entered, a date/time stamp will be appended to the "Sample" default (e.g., Sample 2024/03/25-10:58:09).*
2. Use the **Select Assay** dropdown to choose an assay. To view and/or edit assay details, tap the **View** button. Tap **Back** (if no changes were made) or **Save** (to save any changes) to return to the Setup screen.
3. In the **Skip Preview** field, select *No* to enable the **Preview** button or *Yes* to skip previewing the sample and proceed to performing a **Count**.
4. In *Consumable Details* area, choose **Type** and **Images Per Well**. *The slide visualization depicts number of Images Per Well (i.e., 2, 4, or 8). Each image represents an area in the counting chamber (or well).*
5. If desired, expand the *Reports and Exports* area to modify the default auto export location.
6. To run the selected assay, insert the slide.

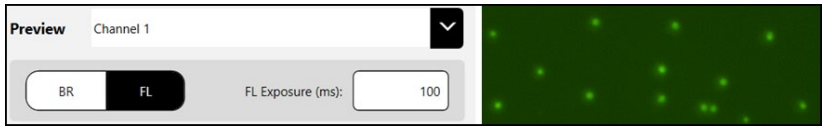
Preview Mode

The Preview screen displays live images for selected areas in the well. *Preview mode will be enabled if **Skip Preview** field is set to No.*

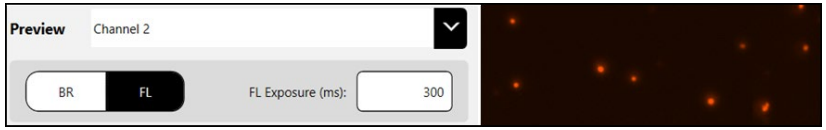
1. Expand the *Details* area to view selected assay and consumable ID.
2. In *Consumable* area, tap a well.
3. In *Areas* visualization for the selected well, tap an area location on the slide. The live image displayed changes as you move from one area to another.



   To zoom in and out of an image, 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.) To move a zoomed image around, tap and drag to a new location.
4. Adjust focus by tapping the **Auto Focus** button.
5. In the *Preview* area, channels available for viewing are based on the assay Imaging Mode. The *Channel 1/BR* image is displayed by default. Tap the **FL** button to display the *Channel 1/FL* image.

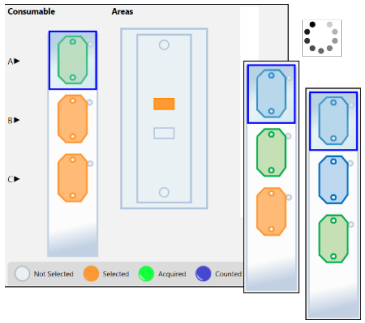


6. To view images for *Channel 2*, select it from the **Preview** field dropdown. *The Channel 2/BR image will be displayed by default.* Tap the **FL** button to display the *Channel 2/FL* image.



7. When previewing fluorescent images, confirm that FL signal is strong but has a low, dark background. Modify the **FL Exposure** to increase (prolong) or decrease (shorten) the exposure time accordingly.

Count Mode



Once you have completed previewing live images for the samples, tap **Count** to acquire images and analyze results. *Count mode will be enabled if **Skip Preview** field is set to Yes. Instrument will automatically perform a count after a slide is inserted.*

As the system acquires sample images, the colors used to mark well slide chambers 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 well. Additional tabs may be enabled for the scan result (e.g., **Summary**).

Analyzing Scan Results

Scan results are displayed (using assigned report templates/tabs), and/or printed and exported based on assay *Reports and Exports* settings.

When analyzing scan results, an image is displayed in the viewing pane for the selected area of the counting chamber. Tap on other chambers or chamber areas to view additional images.

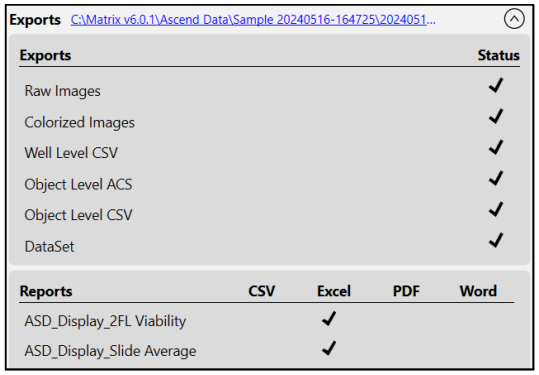
1. In *Areas* visualization, tap an area to display its image in viewing pane.
2. At the top of the **Well View** tab, tap the *Brightfield* (**BR**) or *Fluorescence* (e.g., ***FL1*** and ***FL2***) buttons to select/de-select channels used in display of the image. *Channel views are overlaid on top of each other.*
3. Tap the **Counted Overlay** button to show 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).*
4. Tap the **Zoom** button to enable display of *Zoom* magnification in bottom right corner of viewing pane.

At the bottom of the **Well View** tab is a report containing well-level details for the selected chamber. To view a full report containing results for all acquired and counted chambers, tap the **Summary** tab.

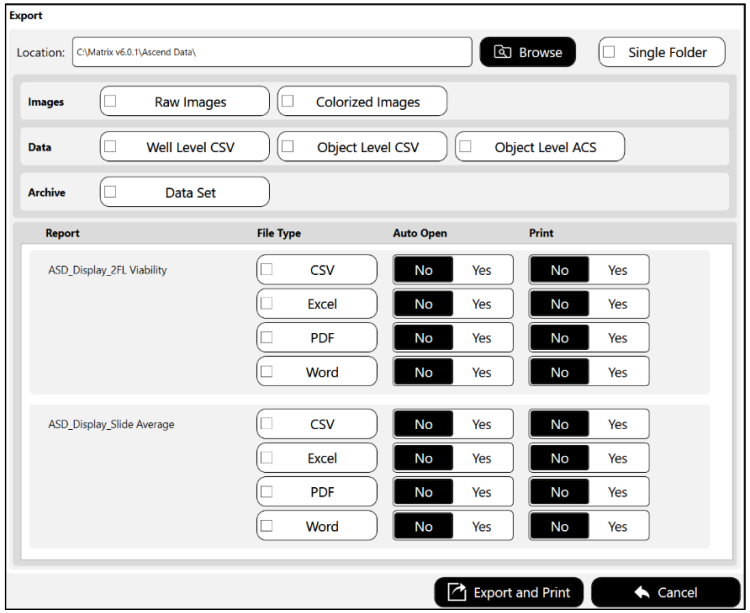
Verifying Auto Exports

Expand the *Exports* area in the Results screen to see completed automatic exports (indicated with checkmarks) and output file types (e.g., *CSV*, *Excel*, *PDF*, and *Word*) as defined for the selected favorite or assay. Tap the **Exports** link to open the folder where exported files are located.

To modify default *Reports and Exports* options defined for an assay during a count or recount, tap the **View** button and expand the *Reports and Exports* area for that assay. Make modifications as needed and then save your changes to the assay or save the changed assay with a new name.

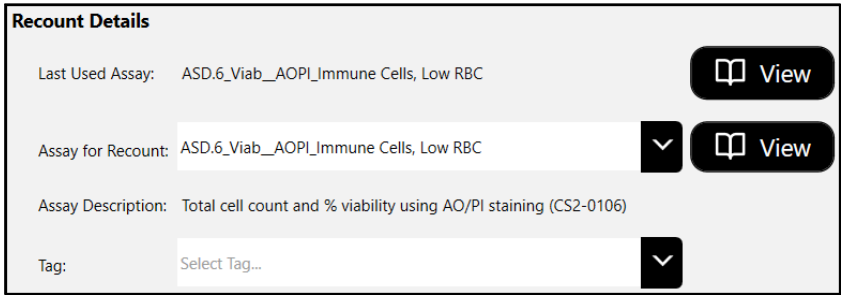


An alternative to modifying default *Reports and Exports* options for an assay is to perform a manual export *after* image acquisition by tapping the **Export** button at the bottom of the Results screen and selecting additional options.



Performing a Recount

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

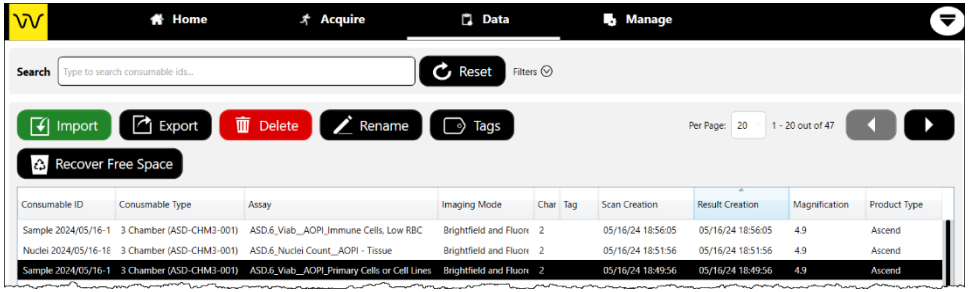


In the *Recount Details* area, view parameter settings for the *Last Used Assay* or select a new *Assay for Recount* and view/edit its parameters.

To view/edit channel mappings in a recount, expand the *Channel Mappings* area to identify *Scan Channels* and *Recount Channels* for assay Imaging Mode. Drag a mapping indicator to a new channel. If there is already a mapping indicator in that channel, it will swap positions with the one you just dragged to that location. *To return channel mappings to their original positions, tap the **Reset** button followed by **Yes** to confirm the action.*

Viewing the Data Tab

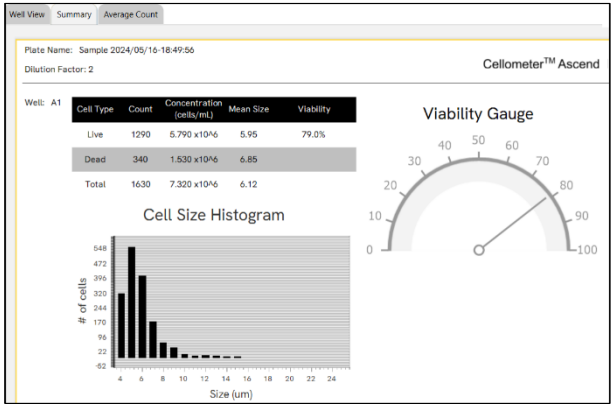
As a continuation of the data acquisition workflow, the **Data** tab contains the *Select*, *Results*, and *Recount* screens which must be completed in sequence when analyzing scan results.



To search for a scan result, enter a few characters from a Consumable ID into the **Search** field and/or expand the *Filters* area to specify search criteria. *You may need to collapse the Filters area to view search results.*

To open a scan result contained in the *Results List*, either double-tap the result or tap it once (to highlight it) and then tap the **View** button.

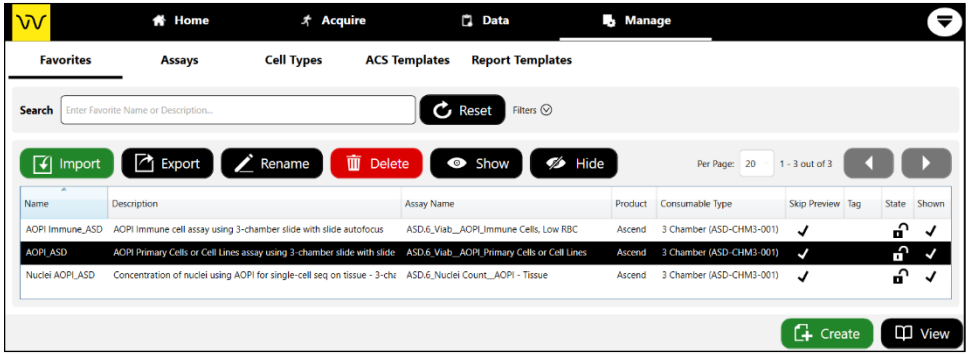
The scan result opens in the Results screen and captured images are shown in the **Well View** tab. You can change the image displayed by selecting different slide areas, and tapping BR/FL channel buttons. Tap the **Summary** tab to view a summary report of results for all chambers.



Viewing the Manage Tab

The **Manage** tab contains the *Favorites*, *Assays*, *Cell Types*, *ACS Templates*, and *Report Templates* options. Tap an option to view complete listings of these entities loaded in your database. For each option you can import/export, rename, delete, or show/hide any entity in their respective list.

The *State* column displays icons indicating whether an entity is *Locked* or *Unlocked*. Although a locked favorite, assay, cell type, or report template cannot be edited, you can select it to use as a source for creating a new entity and by tapping the **Save As** button to save it using a new name.



To search for a favorite, assay, cell type, ACS template, or report template, enter a few characters from an entity name into the **Search** field and/or expand the *Filters* area to specify search criteria. *You may need to collapse the Filters area to view search results.*

ACS templates can be imported into the Matrix software and assigned to an assay. On export, if the *Auto Open* option is selected and De Novo Software *FCS Express* is installed, the application will be launched and scan result data is automatically populated into the specified ACS template.

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 your detailed needs and we can help support the creation of new templates.

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 2.0 x 10^4 – 2.0 x 10^7 cells/mL can be analyzed on the Ascend, with an optimal concentration of ~2.0 x 10^6 cells/mL.

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 or any cell resuspension.*

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.

To measure viability, stain cells by combining 20 µL* of cell sample with 20 µL* of a 0.2% trypan blue staining solution (for a final concentration of 0.1% trypan blue with cells). Gently mix by pipetting up and down 10x before adding sample to a counting chamber.

* Volumes noted above are optimized for 3-chamber slides. If you are using 8-chamber slides, reduce cell sample and stain volumes to 10 µL each.

Trypan Blue Staining Solution Guidelines

Use the following trypan blue staining solution guidelines to prepare cell samples.

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

Using AO/PI Viability Method

A dual-fluorescence *AO/PI Viability Method* has been developed to more accurately determine nucleated cell concentration and viability in primary cell samples containing debris and non-nucleated cells, including platelets and red blood cells.

In this 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*).

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

To measure viability, stain cells by combining 20 µL* of sample with 20 µL* of AO/PI staining solution. Gently mix by pipetting up and down 10x before adding sample to a counting chamber.

* Volumes noted above are optimized for 3-chamber slides. If you are using 8-chamber slides, reduce cell sample and stain volumes to 10 µL each.

Note: For whole blood and other viscous samples, draw sample in and out of the pipette tip at least once prior to transferring for staining.

The following table shows recommended dilutions when prepping samples for AO/PI viability and dilution factor to enter for a few sample types.

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

* *Sample Volume* and *AO/PI Volume* are optimized in the table above for 3-chamber slides. If you are using 8-chamber slides, reduce *Sample Volume* and *AO/PI Volume* to 10 µL each.

AO/PI Staining Solution Guidelines

Use the following AO/PI staining solution guidelines to prepare cell samples.

Stain Type	***Use with Cell Sample***	***Dilution Factor***
AO (CS1-0108-5mL)	1:1	2
PI (CS1-0109-5mL)	1:1	2
AO/PI (CS2-0106-5mL)	1:1	2
AO/PI (CS2-0106-25mL)	1:1	2

Available Product Documentation

See the following documentation for additional instrument information:

- ***8004707 Cellometer Matrix Software User Manual*** for complete software functionality details (available as a PDF on USB Drive).
- ***8004708 Cellometer Ascend User Manual*** for instrument operation, care, and maintenance details (available as a PDF on the USB Drive).
- ***8004710 Cellometer Ascend Focus Guide*** for assistance in obtaining 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.

