Cellometer® Auto 2000 Quick Start Guide

1. **Current Assay Indicator** – Displays currently selected assay.
2. **Edit Assay Button** – Enables users to customize parameter settings for the currently selected assay and associated cell types.
3. **Import Assay Button** – Enables users to import and export assays.
4. **Sample ID Input Field** – Allows entry of a unique sample identifier.
5. **Dilution Factor Input Field** – Allows entry of final dilution factor.
6. **Assays Available for Selection List** – Displays all assays available for analyzing samples. The current assay is highlighted with a red outline.
7. **Last Count Results Icon** – When enabled, displays last available count results.
8. **Left/Right Arrows** – Enables users to scroll left/right to view all assays.
9. **Preview Icon** – Displays a preview image for the currently selected assay.
10. **Load Icon** – Loads previously saved sample images for analysis.
11. **Settings Icon** – Enables users to edit counting/saving options and establish a background image for the instrument.
12. **Help Icon** – Provides users with access to online Help and Support resources.

Contents of Shipping Container

☐ **Cellometer Auto 2000 Instrument**

☐ **Power Supply** and **Power Cord**

☐ **USB Drive** – Contains **Cellometer Auto 2000 Software**

☐ **Cellometer Auto 2000 User Manual** and **Quick Start Guide** – PDF files stored on USB Drive

☐ **Focus Guide** – Graphic sheet intended to help users adjust instrument focus

☐ **Counting Chamber Slides** – One box of 75 disposable slides; two counting chambers per slide

☐ **Fluorescent Beads** and **AO/PI Viability Reagent**

Unpacking the Instrument

Unpack and visually inspect the Auto 2000 to ensure no physical damage has occurred during shipping. For assistance in setting up the instrument, visit the Cellometer Auto 2000 page on our website for training videos on unboxing and getting started.

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 Auto 2000.

Keep the area around the instrument clean between, during and post operation. Do *not* position the device so that it is difficult to disconnect from power main.

Using Instrument for the First Time

As the Auto 2000 is a touch-screen device, tap gently on screen elements (e.g., icons or buttons) using a finger or stylus to interact directly with software GUI options. *As an alternative, a USB mouse may be connected.*

Starting the Auto 2000

Plug power cord into an outlet and press the Power Switch on front of the instrument to display as blue (indicates *Run* mode). *Button displays as orange while instrument is in Standby mode.* The Auto 2000 software GUI will load automatically.

Taking a Background Image

After initial setup of the instrument, you must take a background image of the system by itself (without the counting chamber slide inserted) to normalize the cell counter. Unless the instrument is moved to a new location, there is generally no need to take another background image.

1. Tap the **Settings** icon located on bottom panel of the Home screen.
2. In the *Instrument Settings* area, tap the **Take Background Image** icon.
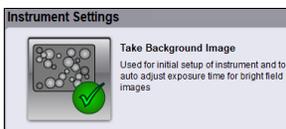
3. Remove slide from instrument, if applicable. Tap **Continue** in response to pop-up message to confirm Auto Sample Slot is empty.
4. The system takes the background image and displays a pop-up indicating when the image has been saved. Tap **Continue**.
5. Tap the **Preview** icon located on bottom panel of the Home screen.
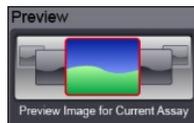
6. Confirm that background is a uniform gray color. If there is any discoloration (e.g., light/dark areas) of the image, contact Support via email: CellC-support@revvity.com

Setting Up Auto Save Feature

1. Insert a USB drive into the instrument's USB port or connect an Ethernet cable to link instrument to a network.
2. From the Home screen, tap the **Settings** icon.
3. In the *User Settings* area, tap the **Saving Options** icon.
4. In the *Auto Save* area, tap both **Auto Save to Data File** and **Save Raw Images** buttons to display as *ON*.

Only raw images can be loaded into the Auto 2000 for reanalysis or sent to Support when requesting assistance with optimization of assay/cell type parameters.

5. Tap each of the **File:** *file_name* and **Folder:** *file_path* buttons to specify the file name and file path for automatically saving count result data and images. If necessary, create a new folder and/or data file. *Default file name may be data.txt (i.e., plain text with delimiter-separated values) or data.csv (i.e., plain text with comma-separated values). Separating data using either delimiters or commas prepares it for use in spreadsheets.*
6. Tap **Done** to save options, then **Done** again to return to Home screen.

Staining Solution Guidelines

Use the following staining guidelines to prepare cell samples for Auto 2000.

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

See *Evaluating Viability Methods* on page *6* for details on how to choose a staining method based on the cell sample and selected assay type.

Preparing Sample Slides

Although the Auto 2000 does not require any routine testing or calibration, counting beads are available to verify instrument functionality. *Revvity counting beads CCBM-011-2ML are recommended for use with Auto 2000.*

To prepare slides containing counting beads or cell samples:

Top of Slide

1. 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 the film from the slide.
2. Place the slide on a fresh Kimwipe.
3. To prepare two samples at once, label individual chambers (e.g., *Chamber 1* and *Chamber 2*) in the white margin. Take care to ensure that the clear optical windows of the counting chamber are *not* touched.

Bottom of Slide

4. 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.*
5. Set pipette to 20 μL and then pipette bead solution/cell sample up and down ten times (10x) to break up any potential clumps.
6. Load 20 μL of counting beads or cell sample into counting chamber of Cellometer slide (CHT4).
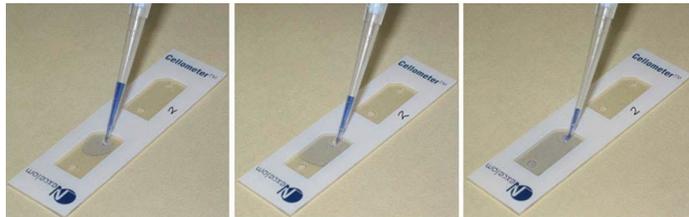
7. Hold the loaded chamber in the white area and insert counting chamber containing sample into Auto Sample Slot until touching the internal stop.
8. Select an appropriate assay based on cell type and tap the **Preview** icon.

Note: Cell concentrations of $1.0 \times 10^5 - 1.0 \times 10^7$ cells/mL can be analyzed on the Auto 2000, 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.

Adjusting Instrument Focus

While previewing a sample slide loaded into the instrument, icons are displayed for the **Select Image to Preview** field based on the *Imaging Mode* defined for the current assay.
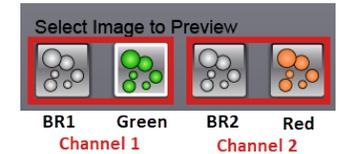
Select a brightfield image (e.g., *BR1 if two brightfield images are displayed*) and tap gently on the *Coarse Focus* or *Fine Focus* controls located on the left side of Preview screen. Proper focus is key to ensuring accurate cell counts.

Counting beads will appear as dark circles with sharp edges.
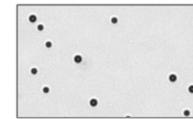
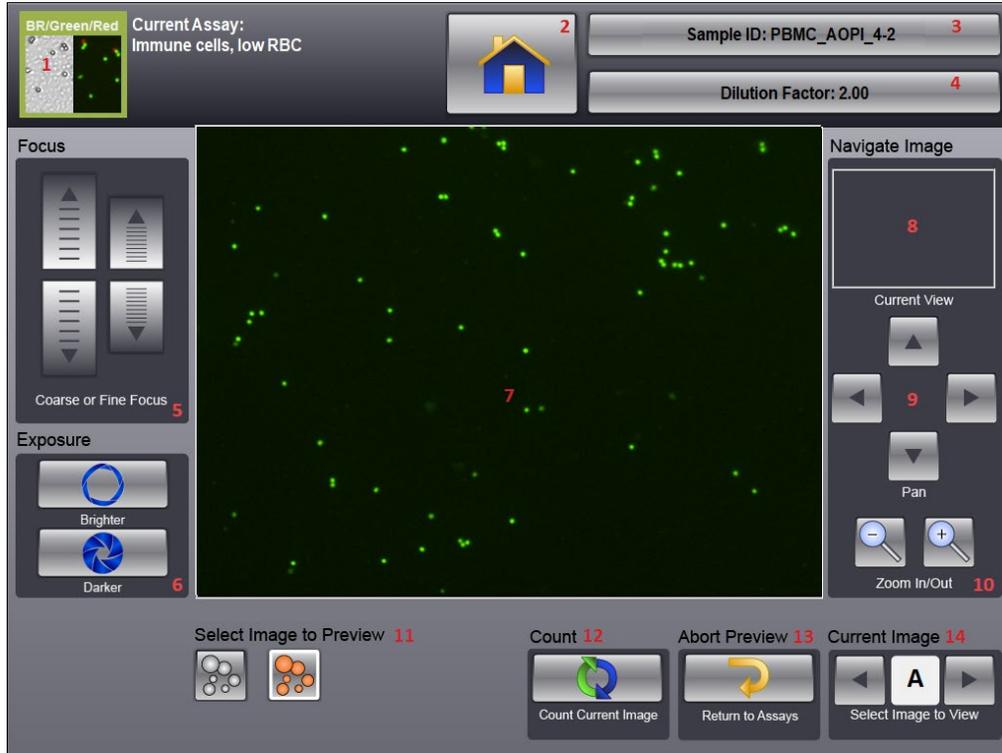Live cells should have a bright center and dark, crisp clearly defined edges.
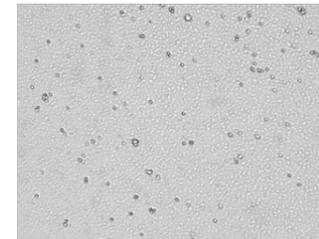
If fluorescent images are displayed, tap the *Green* and/or *Red* image icon. Confirm fluorescent spots have sharp edges. If the edges appear to be fuzzy, adjust focus and fluorescent exposure time (using the **Exposure** controls) as necessary. If a second fluorescent image is available, only the fluorescent exposure time may need to be adjusted.
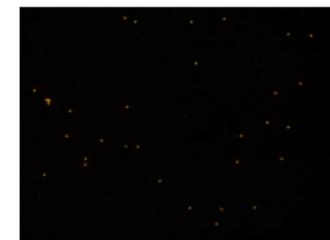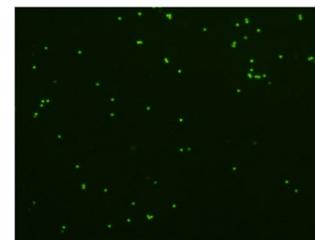
Once good focus is achieved, the instrument should perform most counting operations with only minor adjustments. You are now ready to begin automated cell counting using the Auto 2000.
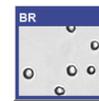
Counting and Analysis Workflow

13 **Abort Preview Icon** – Cancels current preview and returns to Home screen.

14 **Current Image Control** – Switches to images A, B, C and D of sample.

After you have adjusted instrument focus in the Preview screen, you are ready to begin automated cell counting and analysis.

1. Tap on the **Sample ID** input field, enter a sample name, then tap **Save**.
2. Tap on the **Dilution Factor** input field to enter the final dilution factor for the sample, then tap **Done**.
3. Tap the **Count** icon to display the Counting screen used to monitor counting progress. *Display a progress bar and numeric counter.*
4. When counting is complete, the Count Results screen is displayed. Tap the **Sample** icon at the bottom of the screen to calculate sample adjustment or the **Details** icon to observe sample images.

1 **Current Assay Indicator** – Displays currently selected assay.

2 **Home Icon** – Returns to Home screen for assay selection.

3 **Sample ID Input Field** – Allows entry of a unique sample identifier.

4 **Dilution Factor Input Field** – Allows entry of a final dilution factor.

5 **Focus Control** – Refines fine/coarse adjustment of current sample preview.

6 **Exposure Control** – Adjusts exposure time of current sample preview.

7 **Viewing Pane** – Displays area of sample in *Current View* for *Current Image*.

8 **Navigate Image Control, Current View** – Displays red rectangle indicating area of sample being displayed. *Moves with Pan and Zoom In/Out controls.*

9 **Navigate Image Control, Pan** – Moves area of sample being displayed (indicated by red rectangle in *Current View*) in the direction of arrows selected.

10 **Navigate Image Control, Zoom In/Out** – Zooms in or out of sample image being displayed (indicated by the red rectangle in *Current View*).

11 **Select Image to Preview Icons** – Displays icons for images available to be previewed based on the *Imaging Mode* defined for the current assay.

12 **Count Icon** – Initiates counting of the sample.

Default Cell Line Assays

Cell Line, Total Cell Concentration
No Stain; Cell line or primary cells without debris, with viability greater than 98%.

After taking brightfield images of a sample, all cells are counted to determine total cell concentration.
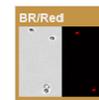
Cell Line, Viability Propidium Iodide
PI (CS1-0109); Cell line or cultured primary cells without debris.

Propidium iodide (PI) is routinely used to determine cell viability. PI is a fluorescent stain that only penetrates dead cells and emits in the 'red' range (live cells are unaffected by PI).

After taking both brightfield and 'red' fluorescent images of a PI stained sample, all cells (from brightfield channel) and dead cells (from 'red' fluorescent channel) are counted to determine total and dead cell concentrations. Counts are then used to calculate percent viability.

Cell Line, Viability Trypan Blue
*Trypan Blue; Cell line or cultured primary cells without debris.*

Trypan blue is routinely used to determine cell viability. Trypan blue penetrates and stains dead cells while leaving live cells unstained.

After taking brightfield images of a trypan blue stained sample, live and dead cells are counted to determine total, live and dead cell concentrations. Counts are then used to calculate percent viability.

Sample Adjustment Tool

Available in the Count Results screen, tap the **Sample** icon to activate the *Sample Adjustment* tool to assist you with calculating the adjustment necessary to achieve a specified target concentration or target number of cells.
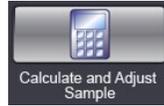
Cell Size Analysis Tool

Available in the Count Details screen, tap the **View Size Histogram** icon to activate the *Cell Size Analysis* tool displaying cell size data from count results. This tool generates a histogram that enables users to optimize cell diameter parameters for the sample (e.g., to exclude debris or very large cells) and refine count data.
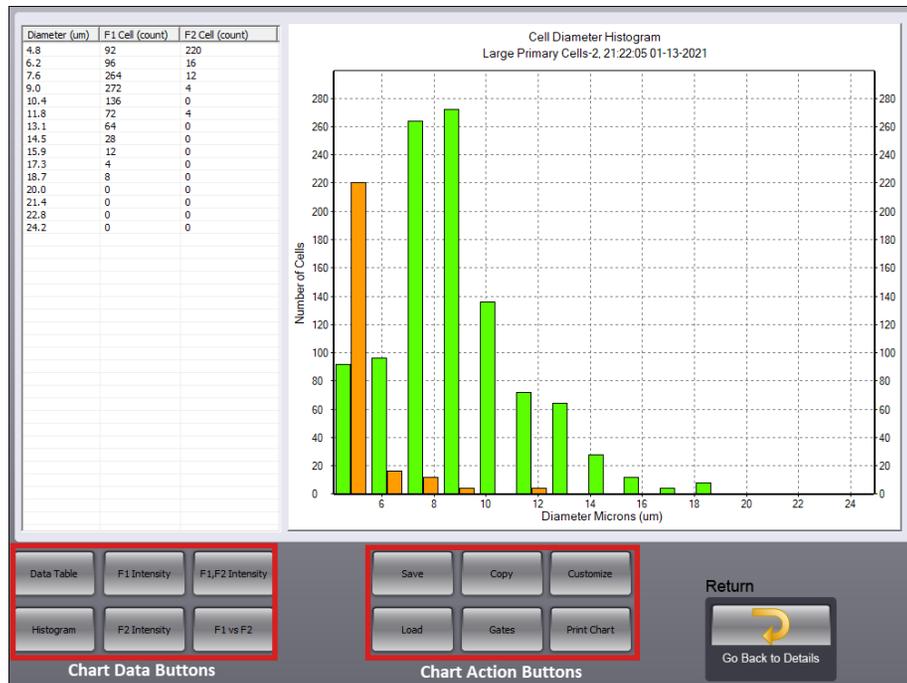

Tap any of the *Chart Data Buttons* to display the full data table created by the tool or other view chart variations showcasing key aspects of the data. Tap the *Chart Action Buttons* to save, copy, print or customize the chart.

## Viewing Counted/Combined Images

Available in the Count Details screen, tap the **View Counted Image** icon to view the counted image while displaying a graphic Overlay that highlights counted cells with a green outline. This overlay may also display a red outline around counted dead cells (if testing for cell viability) or a yellow outline around cells *not* counted as they are larger than the specified cell diameter.

After selecting the **Details** icon to observe cell images, tap the *Green* and/or *Red* fluorescent image icons in the bottom panel, followed by the **View Counted Image** icon on the left of the screen to view counted cells. Enlarge the image by tapping the **Zoom In (+)** icon. For the *Green* image, confirm that all the live (green fluorescent) cells are circled in green and that for the *Red* image, all the dead (red fluorescent) cells are circled in green.
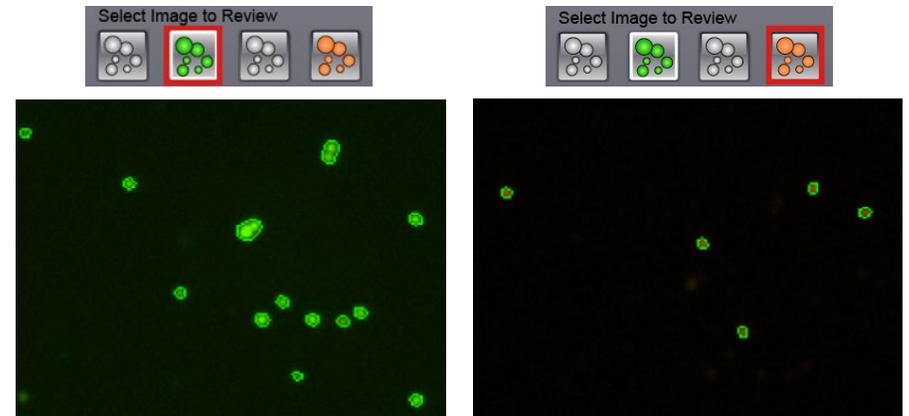
Tap the **View Combined Image** icon to view both *Green* and *Red* flourescent images together. In addition, tap the **View Counted Image** icon to toggle display of the Counted graphic overlay.
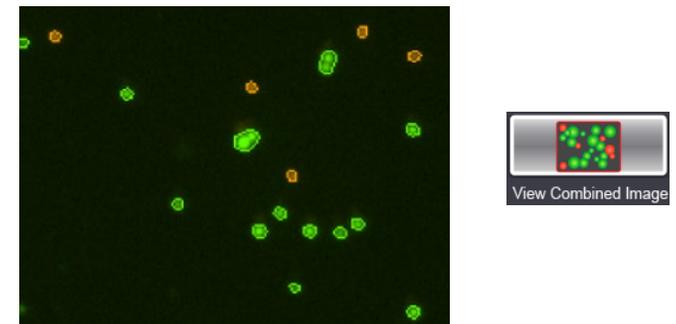
Evaluating Viability Methods

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 Auto 2000, 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.

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 Auto 2000, 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 20 μL of cell sample with 20 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 chamber (CHT4).

The table below shows the recommended dilution when preparing cell samples for AO/PI viability analysis and the final *Dilution Factor* to enter into Auto 2000 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	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

Default Primary Cell Type Assays

In the Auto 2000 Home screen, select an assay based on the primary cell type being analyzed. Enter a **Sample ID** and **Dilution Factor**, accounting for any other sample dilutions. The following four assays are pre-optimized with specific counting parameters for the cell type(s) indicated below.

Immune Cells, High RBC

AO/PI (CS2-0160) or equivalent; Nucleated cells in samples with large amount of red blood cells. No RBC lysing.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images of an AO/PI stained sample, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

Immune Cells, Low RBC

AO/PI (CS2-0106) or equivalent; Nucleated immune cells after isolation in samples with some red blood cells. PBMC after ficoll separation, splenocyte without lysing RBC.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

Primary Cells, Cell Lines

AO/PI (CS2-0106) or equivalent; Primary cells, cell lines or cell sample from dissociated tissue with debris.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells.
Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

Stem Cells

AO/PI (CS2-0106) or equivalent; Stem cell sample.

Acridine orange (AO) is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide (PI) is a fluorescent stain that only penetrates dead cells and emits in the 'red' range.

After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted to determine concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells. Counts are then used to calculate percent viability.

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.

See the following documentation for additional instrument information:

- ***8001334 Cellometer Auto 2000 User Manual*** for instrument operation, care and maintenance details (available as a PDF file on the USB Drive)

- ***8001164 Cellometer Auto 2000 Focus Guide*** for assistance with optimal focus