



STEM CELL LABORATORY (STCL)



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Cellometer® Auto 2000

User Manual

Cellometer Auto 2000 User Manual

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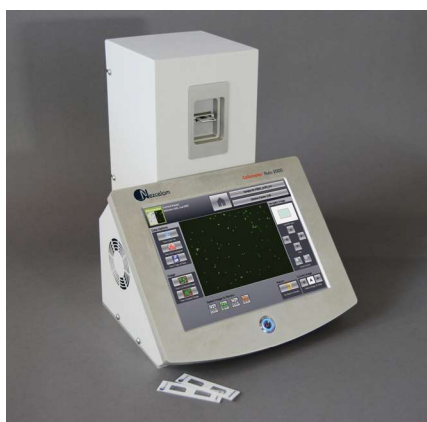
Introduction

What is Cellometer Auto 2000?

Cellometer® Auto 2000 is a compact, automated cell counting system utilizing dual fluorescence to detect, count, measure cell size and calculate cell concentration from a 20uL cell sample. The basic principle of the Cellometer automatic cell counter is imaging cytometry. Cells are loaded into the Disposable Counting Chamber and automatically spread into a thin layer by capillary action. Cellometer Auto 2000 then captures images of cells in the counting chamber, analyzes the number of cells, sizes and fluorescence intensity of each cell, and then converts this data into concentration, size and viability.

The Cellometer Auto 2000 system consists of 2 main components:

1. Cellometer Auto 2000 instrument with analyzing software



2. Disposable Counting Chambers



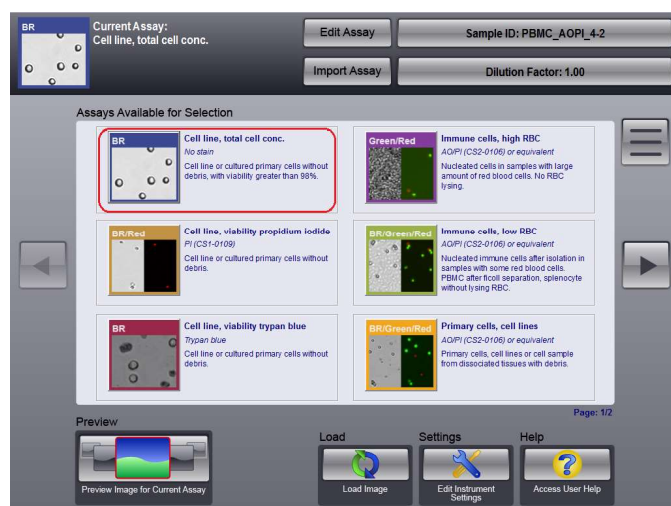
Disposable counting chamber accommodates 2 individual samples and can be loaded through either port.

Pipette 20uL of cell sample into one of the ports with any standard single channel pipette.

Cellometer Auto 2000 comes with a starter set of 75 slides. Slides can be ordered directly from Nexcelom or your authorized Nexcelom dealer.

Quick Operation Instructions

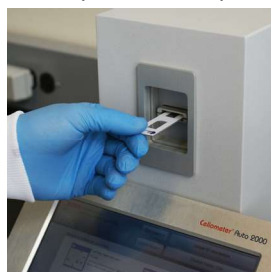
1. Select an Assay



2. Input Sample ID and Dilution Factor



3. Prepare Sample, load disposable counting chamber and insert into instrument



4. Click "Preview"



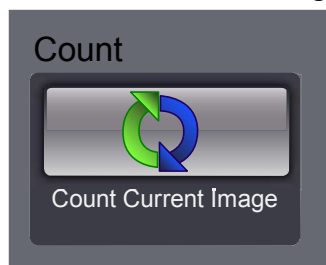
5. Adjust Focus



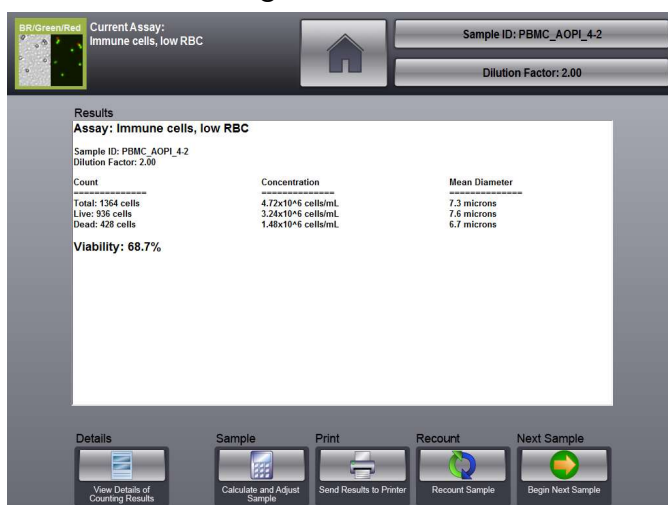
6. Select Green and Red images to preview and adjust exposure if needed



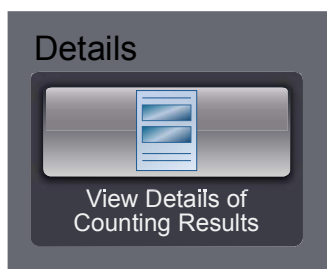
7. Click "Count" to begin counting process



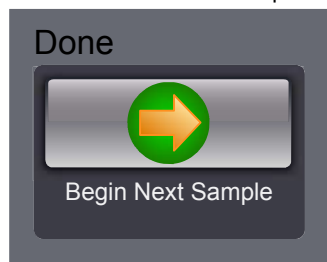
8 .Review Counting Results



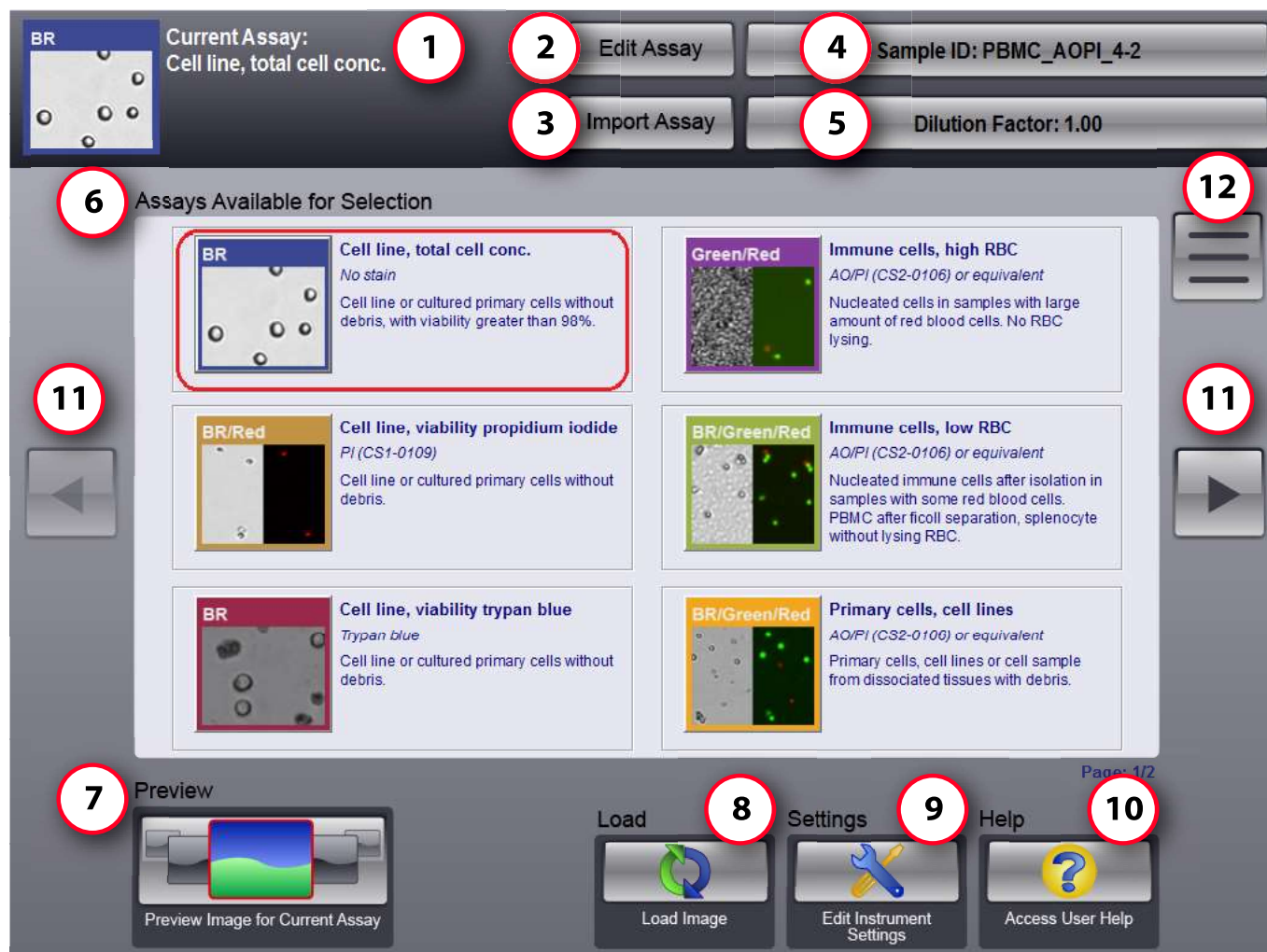
9. Select Details to review cell images and counted cell images



10. Select Next Sample or Assay and Settings when done

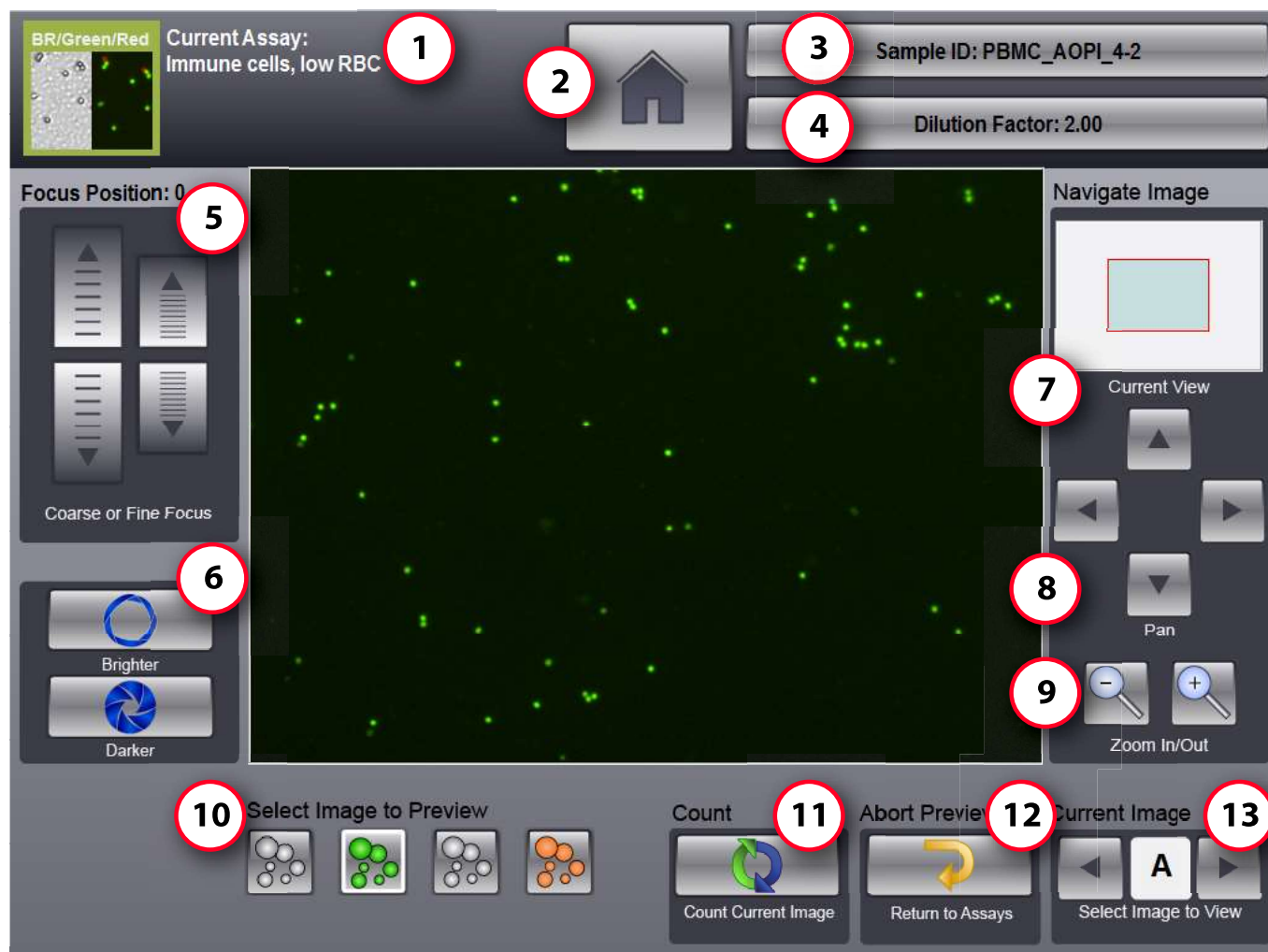


User Interface - Assay Selection Screen



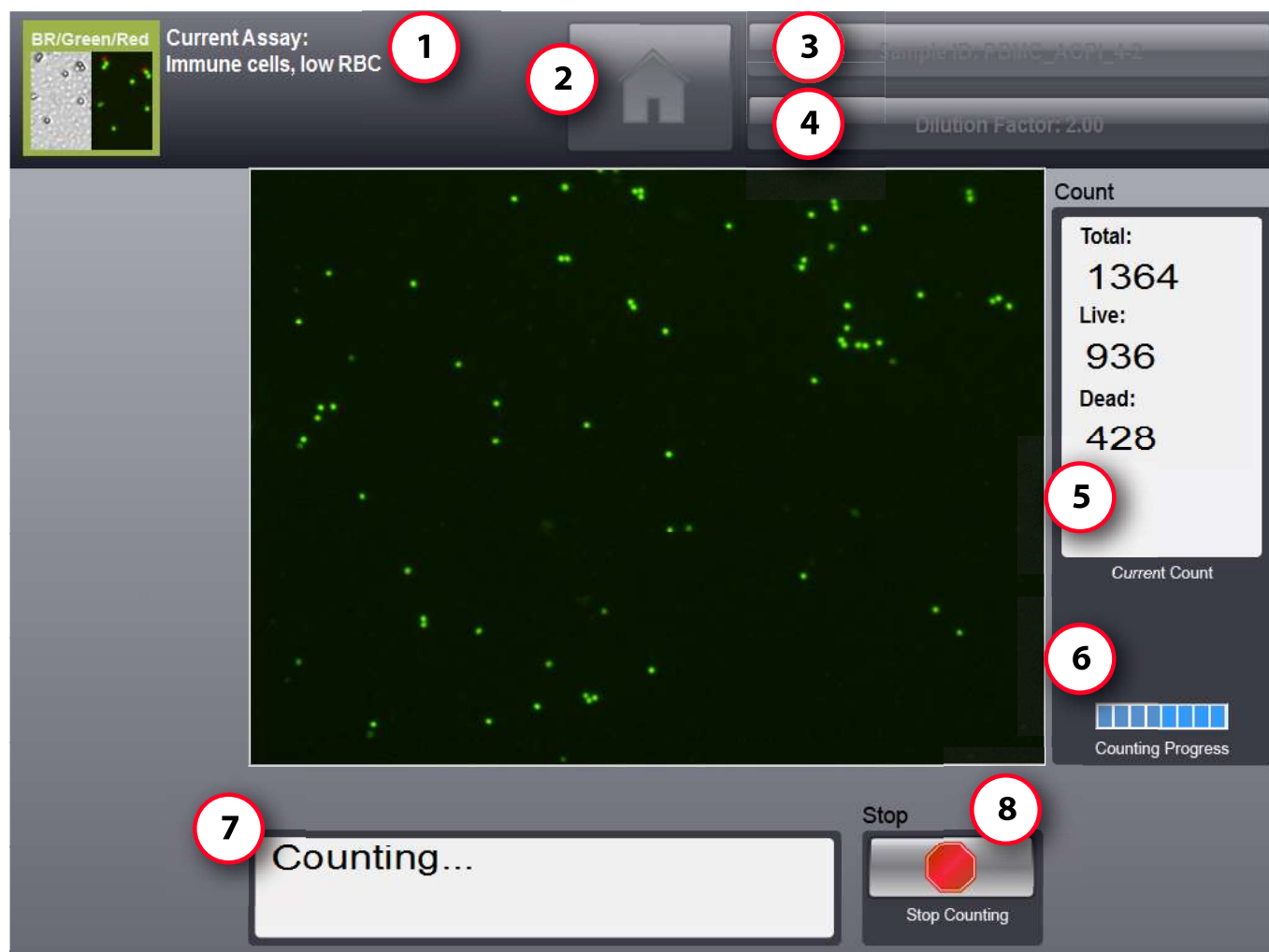
1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Edit Assay** Select to edit the Assay and Cell Type settings
3. **Import Assay** Select to Import additional assays
4. **Sample ID** Select to enter a Sample ID
5. **Dilution Factor** Select to enter a Dilution Factor
6. **Assays Available** List of Assays available for selection
7. **Preview** Select to Preview the cell sample image
8. **Load** Select to load and analyze previously saved images
9. **Settings** Select to edit instrument and UI settings
10. **Help** Select to access help options
11. **Left/Right Arrows** Select to page left/right to access all assays available
12. **Return to Results** Select to return to previous count results

Preview Screen



1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Home** Select to return to Assay Selection Screen
3. **Sample ID** Select to enter a Sample ID
4. **Dilution Factor** Select to enter a Dilution Factor
5. **Focus** Fine and coarse focus adjustment of the current cell image
6. **Exposure** Adjust the exposure time of the current cell image
7. **Current View** Red rectangle to show what area of the cell image is currently displayed
8. **Pan** Moves the area currently being shown within the current cell image
9. **Zoom** Zooms in or out of the current cell image
10. **Select Image to Preview** Select brightfield, green or red fluorescence images to preview
11. **Count** Starts counting of the current cell image
12. **Abort Preview** Cancels current cell preview and returns Assay Selection Screen
13. **Current Image** Select location on slide to preview

Counting Screen



1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Home** Select to return to Assay Selection Screen (disabled while count is in progress)
3. **Sample ID** Select to enter a Sample ID (disabled while count is in progress)
4. **Dilution Factor** Select to enter a Dilution Factor (disabled while count is in progress)
5. **Current Count** Shows cell count numbers
6. **Counting Progress** Visually indicates the current count progress
7. **Image Being Counted** Indicates which image is currently being counted
8. **Stop** Stops cell counting

Results Screen

The screenshot shows the 'Results' screen of a cell counting application. At the top, there is a header bar with a 'BR/Green/Red' assay icon (1), the text 'Current Assay: Immune cells, low RBC' (1), a home button (2), a 'Sample ID: PBMC_AOPI_4-2' field (3), and a 'Dilution Factor: 2.00' field (4). Below this is the 'Results' section (5) which displays the assay name, sample ID, dilution factor, and a table of cell counts and viability. At the bottom is a navigation bar with five buttons: 'Details' (6), 'Sample' (7), 'Print' (8), 'Recount' (9), and 'Next Sample' (10).

BR/Green/Red Current Assay: Immune cells, low RBC

1 2 3 Sample ID: PBMC_AOPI_4-2

4 Dilution Factor: 2.00

5 Results

Assay: Immune cells, low RBC

Sample ID: PBMC_AOPI_4-2
Dilution Factor: 2.00

Count	Concentration	Mean Diameter
Total: 1364 cells	4.72x10 ⁶ cells/mL	7.3 microns
Live: 936 cells	3.24x10 ⁶ cells/mL	7.6 microns
Dead: 428 cells	1.48x10 ⁶ cells/mL	6.7 microns

Viability: 68.7%

6 Details 7 Sample 8 Print 9 Recount 10 Next Sample

View Details of Counting Results

Calculate and Adjust Sample

Send Results to Printer

Recount Sample

Begin Next Sample

1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Home** Select to return to Assay Selection Screen
3. **Sample ID** Select to enter a Sample ID
4. **Dilution Factor** Select to enter a Dilution Factor
5. **Results** Displays cell counting results including total cell count, live/dead cell count and viability
6. **Details** Select to go to the Details Screen
7. **Sample Adjustment Calculator** Select to launch the sample adjustment calculator. Useful for sample adjustment to get desired concentration or total cell number.
8. **Print** Send the counting results to a network printer
9. **Recount** Select to recount a set of captured images again after modifying counting parameters
10. **Done** Select to start a preview of a new cell sample using the same Assay settings

Calculate and Adjust Sample Screen

BR/Green/Red Current Assay: Immune cells, low RBC

Sample ID: PBMC_AOPI_4-2

Dilution Factor: 2.00

1 Sample Adjustment Source

Sample Adjust Source

Total Cells ☒ On

Live Cells ☐ Off

Dead Cells ☐ Off

Sample Adjustment

Original Sample Volume **2**

Target **3** Number of Cells ☒ On

Concentration ☐ Off

Measured Sample Concentration

4.93x10⁶ Cells/ml

Total Cell Number in Sample

4.93x10⁶ Cells

Sample Adjustment

Take 405.71 ul of sample. Good for 2 aliquots.

Print

Send Results to Printer

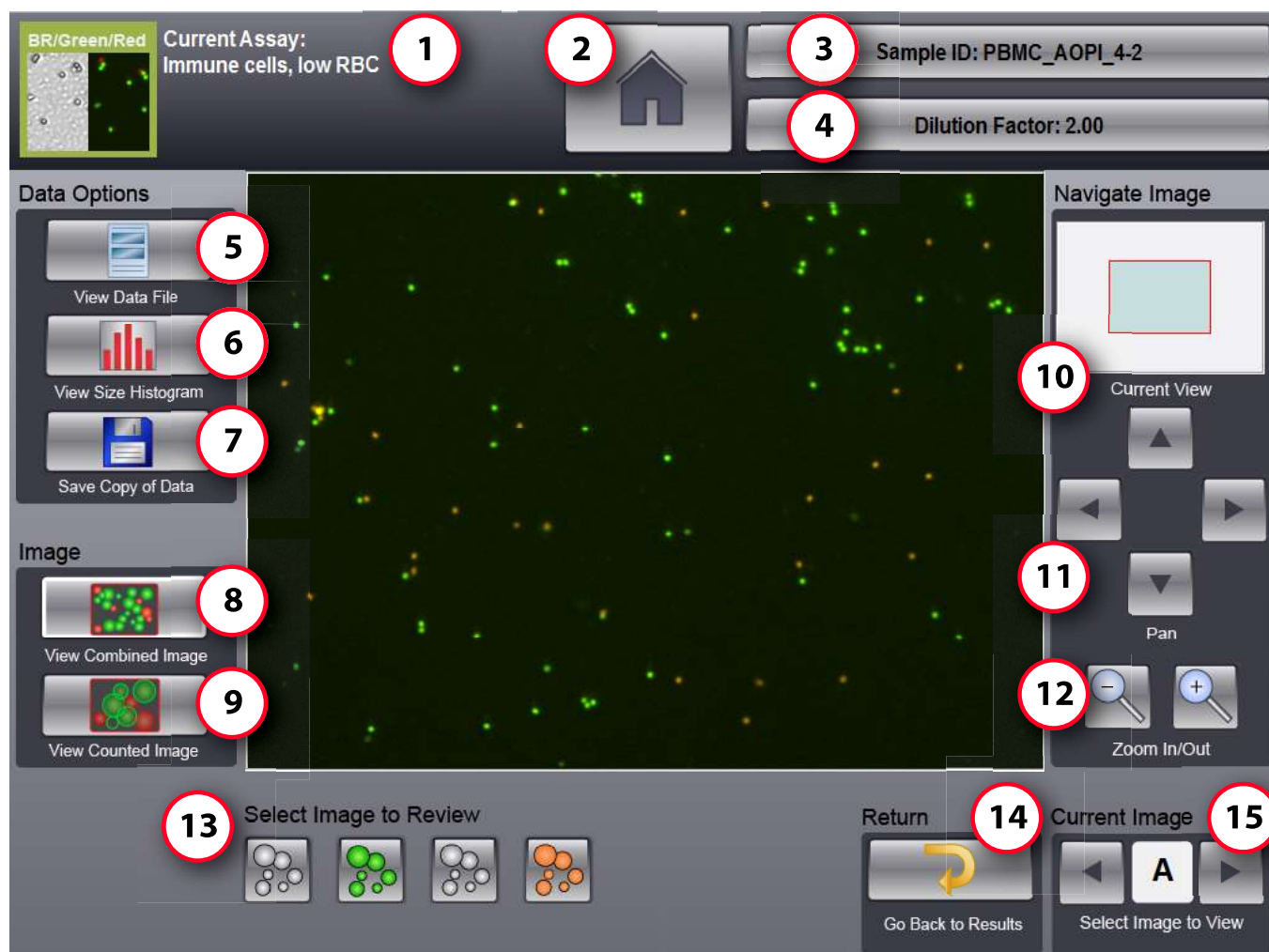
Print with Report ☒ On

Return

Go Back to Results

- 1. Sample Adjust Source** Select which concentration source to use for the Sample Adjustment calculations
- 2. Original Sample Volume** Select to indicate the original sample volume of the cell sample
- 3. Target** Select to indicate the target concentration or number of cells which should be calculated

Details Screen



1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Home** Select to return to Assay Selection Screen
3. **Sample ID** Select to enter a Sample ID
4. **Dilution Factor** Select to enter a Dilution Factor
5. **View Data File** Opens data file to view or print
6. **View Size Histogram** Opens separate window to display cell size histogram
7. **Save Copy of Data** Select to saves the current counting results to the data file
8. **View Combined Image** Cell image showing F1 and F2 fluorescent objects in a single merged image (available only when an assay uses both F1 and F2 images).
9. **View Counted Image** Shows cell image with green and red outlines to indicate cells counted
10. **Current View** Red rectangle indicates what area of the cell image is currently displayed
11. **Pan** Moves the area currently being shown within the current cell image
12. **Zoom** Zooms in or out of the current cell image
13. **Select Image to Review** Select to view the brightfield, Green or red fluorescence counted images
14. **Return** Select to return to Results Screen
15. **Current Image** Select location on slide to view counted image

Assay Parameters Screen


The screenshot shows the 'Assay Parameters' screen. At the top, the title 'Assay Parameters' is centered. Below it, on the left, is a section labeled '1' containing an icon, the assay name 'Immune cells, low RBC', a description 'AQ/PI (CS2-0106) or equivalent', and a detailed description 'Nucleated immune cells after isolation in samples with some red blood cells. PBMC after ficoll separation, splenocyte without lysing RBC.'. To the right of this section is the status 'Unlocked for Editing' and two buttons: 'Edit' and 'Print'. Below this is a section labeled '2' for 'Imaging Mode: Two Fluorescent Stain' with an 'Edit' button. The main area is divided into two columns for 'Channel 1' and 'Channel 2'. Channel 1 is labeled '3' and contains parameters for 'Assay 1 Immune Cells Live' (AO Live Cells, 470 nm light source, 535 nm peak detection) with an 'Edit' button. Channel 2 is labeled '4' and contains parameters for 'Assay 1 Immune Cells Dead' (PI Dead Cells, 540 nm light source, 605 nm peak detection) with an 'Edit' button. Below the channels are 'FL Exposure Time' settings: 600 msec for Channel 1 (labeled '4') and 3.0 sec for Channel 2. At the bottom are 'Advanced Settings' (labeled '5') and 'Calculations and Reports' (labeled '6'), both with 'Edit' buttons. On the far right are 'Cancel' and 'Save' buttons.

1. **Current Assay** Name of assay, description and icon that appears in the Assay Selection screen
2. **Imaging Mode** Select to indicate which type of images are captured and analyzed
3. **Channel 1 and Channel 2** Indicates which Cell Type parameters, light source and detection are used based on the Imaging mode selected.
4. **FL Exposure Time** Select to indicate the exposure time needed for the Channel.
5. **Advance Settings** Select to edit additional settings available for the imaging mode selected.
6. **Calculations and Reports** Select to edit the report formats and the calculations used to generate results.

Assay Description Screen

Assay Descriptions

Assay Icon

BR/Green/Red


Change

Assay Name

Immune cells, low RBC

Description 1

AO/PI (CS2-0106) or equivalent

Description 2

Line1: Nucleated immune cells after isolation in samples with some red bl

Line2: <blank>

Line3: <blank>

Line4: <blank>

Lock Assay from Future Editing: ☐ Off

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.

Demo Image
Auto2K4-PBMC_AOPI_4-1-BR-A.png

Change

Cancel

Save

Imaging Mode Screen

Imaging Mode

Two Stain Fluorescence

Select New Imaging Mode

Total Cell Concentration

Trypan Blue Viability

Single Channel Fluorescence

Brightfield and Single Channel Fluorescence

Two Channel Fluorescence

Dual Channel Fluorescence

Advanced BR/FL Mode ☐ Off ?

Use Viability Mode Reporting On ☒

Channel 1 is Live Channel 2 is Dead On ☒

Channel 2 is Total ☐ Off

Acquire Brightfield Image On ☒

Result Template: <Default>

Print Template: <Default>

Change	Default	Edit	New	Cancel
Change	Default	Edit	New	Save

Channel Settings Screen

Channel 1 Settings

Cell Type: Assay 1 Immune Cells Live
Description:

EditSelect New

Stain: AO Live Cells

Edit

Light Source: 470 nm

470 nm Light Source

540 nm Light Source

Peak Detection: 535 nm

535 nm Detection Peak

605 nm Detection Peak

Cancel

Save

Cell Type Library Screen

Import from Library
☐ Nexcelom Cell Library

Browse...

Cell Types in Cell Library

- 2372
- 2H3
- 3T3
- 786-O
- A204
- A2058
- A3R5
- A498
- A549
- A549 ATCC
- ACHN
- Activated T cell
- Alex cell
- Algae
- AR40
- ARPE-19
- Assay 1 Immune Cells Dead
- Assay 1 Immune Cells Live
- Assay 2 Immune Cells Dead
- Assay 2 Immune Cells Live
- Assay 3 Primary Cells Large Dead
- Assay 3 Primary Cells Large Live
- Assay 4 Cell Line Viability with PI
- Assay 5 Cell Line Trypan
- Assay 6 Cell Line
- Assay 7 Low Conc*

Import Cell >>
Import All >>

Cell Types in Drop-down Menu

- Assay 1 Immune Cells Dead
- Assay 1 Immune Cells Live
- Assay 2 Immune Cells Dead
- Assay 2 Immune Cells Live
- Assay 3 Primary Cells Large Dead
- Assay 3 Primary Cells Large Live
- Assay 4 Cell Line Viability with PI
- Assay 5 Cell Line Trypan
- Assay 6 Cell Line
- Assay 7 Low Conc*
- Assay 8 Primary Cells Large Dead
- Assay 8 Primary Cells Large Live
- x_GFP
- x_GFP viability
- x_Green FL
- x_Red FL
- x_RFP

Delete Cell
Delete All
Save All
Save Cell
Cancel
Done

Current Cell Type: Assay 1 Immune Cells Live

Cell Type Parameters Screen

Cell Type Parameters

Cell Type: Assay 1 Immune Cells Live

Description:

Edit

Cell Diameter (microns) ?

Min Size

4.0

Max Size

20.0

Roundness ?

0.10

Contrast Enhancement ?

0.40

Decluster ?

On ☒

Edge Factor ?

0.5

Th Factor ?

1.0

Advanced Options

Trypan

FL

Print

Cancel

Save

Getting Started

All Nexcelom products undergo a rigorous quality inspection prior to shipment and all reasonable precautions are taken in preparing them for shipment to assure safe delivery.

The instrument should be unpacked and inspected for mechanical damage upon receipt. Mechanical inspection involves checking for signs of physical damage such as scratched, dents, etc.

If damage is apparent, or any components are missing, please immediately contact Nexcelom (+1-978-327-5340 or support@nexcelom.com) or your local dealer.

After unpacking the instrument, plug the Cellometer Power Cable into the back of the instrument. The Auto 2000 is pre-configured with the Cellometer Auto 2000 software. No additional setup or configuration is required.

Starting the Auto 2000

After plugging in the system, simply press the power button on the front of the unit.

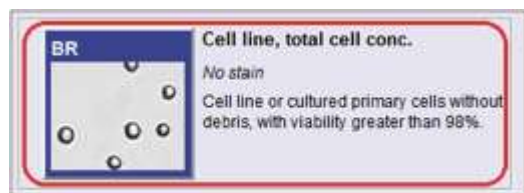


Taking a Background Image

1. When using the Auto 2000 for the first time, a background image should be taken. This step does not need to be repeated unless the instrument is moved.
2. Confirm that there is no slide in the Auto 2000 instrument.
3. Click on the "Settings" button, then take the "Take Background Image" button



4. Check the background image by clicking on the Cell Line, Total Cell Conc. assay and the Preview Image button. The display should show an even white screen. If any dark areas or variations are present, contact Nexcelom Technical Support.



Auto-Save Set-up

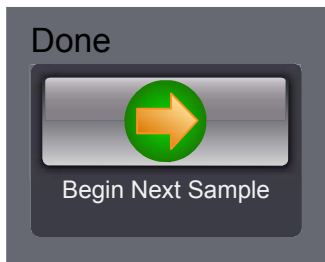
1. Insert a USB drive or connect the Auto 2000 to a network drive via a USB cable.
2. Click on the "Settings" button, then the "Saving Options" button.



3. Click "Auto Save to Data File" and "Save Raw Images" to the "On" position. Click on the File and Folder buttons. In each case, select the USB drive and name the folder. Having saved images will help if any remote technical support is required and provides a permanent record of the raw data.



4. Click "Done", then click "Done" again.

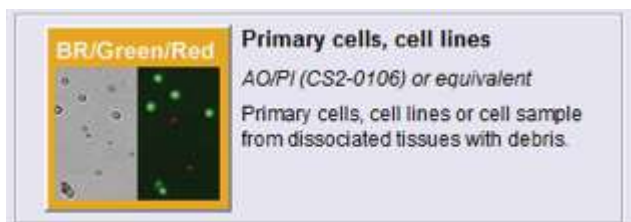


Preparing Reference Beads

1. Though the Auto 2000 instrument does not require any routine testing or calibration, reference beads are available and can be used to confirm fluorescence detection.
2. Cellometer Check Reference Bead Mixture, product number CCBM-006-2ML, is recommended for use with the Auto 2000.
3. Invert the reference bead solution a total of ten times
4. Vortex the bead solution for ten seconds
5. Set pipette to 20 μ l
6. Pipette bead solution up and down ten times to break up any bead clumps

Counting Reference Beads

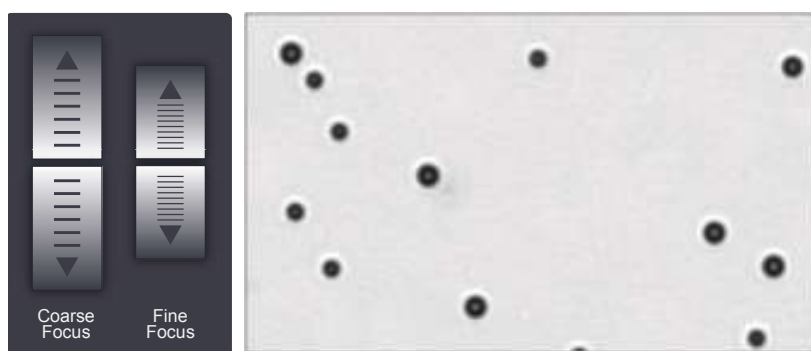
1. Peel plastic off of both sides of the Cellometer slide. (for PD100 slides, the plastic has already been removed)
2. Place cell counting chamber on a fresh Kimwipe
3. Load 20 μ l of mixed Reference Bead Solution into the Cellometer Counting Chamber.
4. Insert the loaded chamber into the Auto 2000 sample slot and gently push the slide to the stop.
5. Select the Primary cell, cell line AO/PI assay.



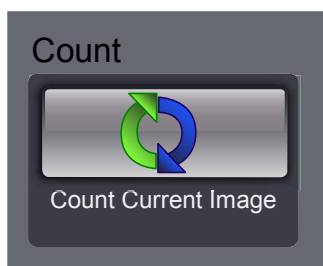
6. Click on "Preview Image for Current Assay"



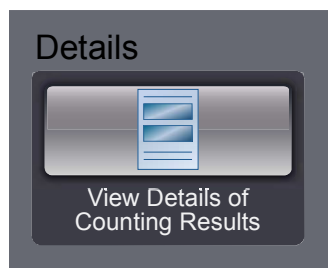
7. Adjust the focus if necessary using the coarse and fine adjustments on the left-hand side of the screen until the best bead counting focus is achieved. The beads should appear as dark circles with sharp edges.



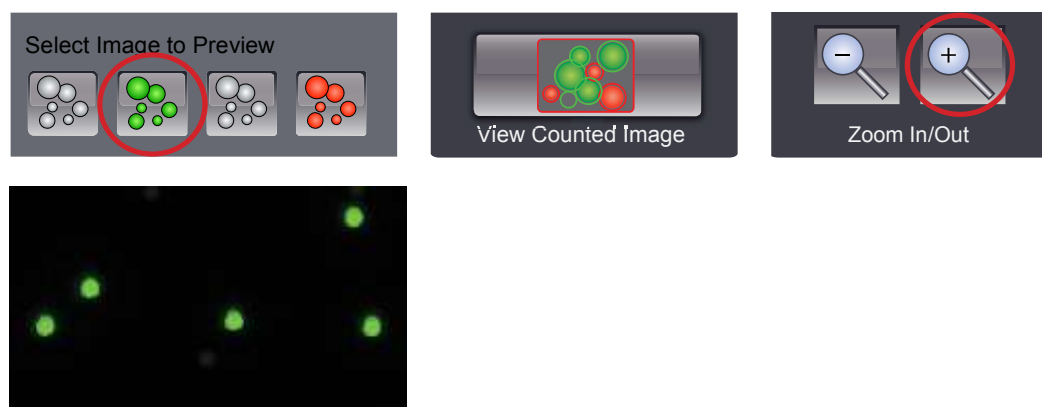
8. Click the Count button at the bottom of the screen.



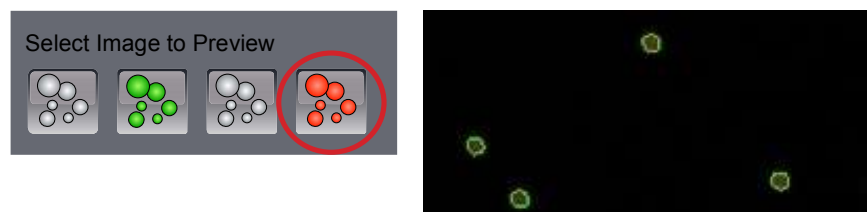
9. When counting is complete, click on the “View Details” button at the bottom left of the screen.



10. Select the green fluorescent image, then click on the “View Counted Image” button on the left-hand side of the screen. Enlarge the image by clicking the “Zoom In” button on the right-hand side of the screen. Confirm that all of the green fluorescent beads are circled in green.



11. Select the red fluorescent image. Confirm that all of the red fluorescent beads are circled in green.



12. Imaging and counting in the bright field, green fluorescent, and red fluorescent channels have now been confirmed.

Comparing Viability Methods

Comparing Methods: When evaluating different viability methods, it is critically important to use one aliquot from the stock cell culture to perform all testing. The cell sample should be evaluated for concentration on the Cellometer Auto 2000 prior to staining. If comparing trypan blue and AO/PI methods, a portion of the tested sample should be stained with trypan blue and a portion should be stained with AO/PI. Starting with the same sample and identical cell concentration enables a more accurate method comparison.

Trypan Blue Viability: For *cell lines and cultured primary cells*, bright field imaging and trypan blue viability may be used to determine the number, concentration, and percentage of live cells. The trypan blue method is *not recommended* for samples containing debris, platelets, or red blood cells. Fluorescence is required to accurately differentiate nucleated cells from platelets, red blood cells, and debris.

AO / PI Viability: Several dual-fluorescence methods have been developed for accurate *nucleated cell concentration and viability determination in primary cell samples containing debris and non-nucleated cells*, including platelets and red blood cells. In the AO / PI method, acridine orange enters all cells and stains DNA, causing all nucleated cells to fluoresce green. Propidium iodide enters all dead cells with compromised membranes and stains the DNA, generating red fluorescence in dead nucleated cells. Cells stained with both AO and PI fluoresce red due to quenching. Live nucleated cells are easily identified in the green fluorescence channel. Dead nucleated cells are easily identified in the red fluorescence channel. There is no interference from debris and non-nucleated cells.

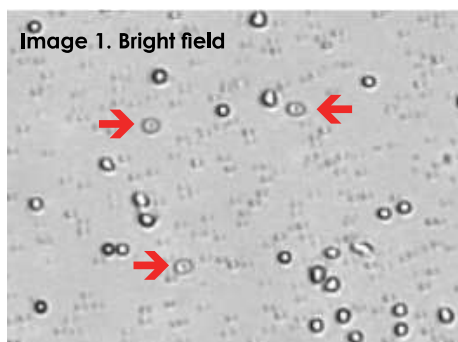


Image 1 and 2. Red blood cells seen in the bright field image are not present in the fluorescent image that is used by Cellometer for AO/PI cell counting. Only nucleated cells are counted, resulting in a more accurate total cell count and % viability calculation.

Preparing Cell Sample for Trypan Blue Viability Determination

1. A cell concentration of 1.0×10^5 to 1.0×10^7 cells/mL can be analyzed on the Auto 2000. A concentration of 1.0×10^6 cells/mL is optimal.
2. Invert the tube containing cells ten times and pipette up and down ten times to generate a homogeneous cell sample and reduce cell clumps. Do not shake or vortex the sample! This will generate bubbles.
3. 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 ten times.

Cell Concentration and Trypan Blue Viability using the Auto 2000

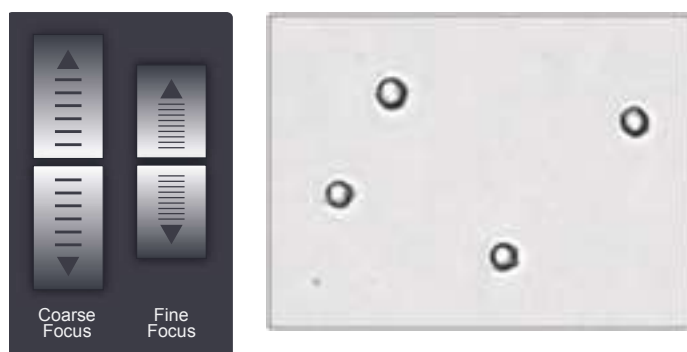
1. Pipette up and down gently 10x to break any potential cell clumps in the stained cell sample
2. Load 20µl of sample into the Cellometer Counting Chamber (CHT4)
3. Insert counting chamber into instrument.
4. Click on the "Cell line, viability trypan blue" assay. When prompted, enter your desired Sample ID and a dilution factor of 2.



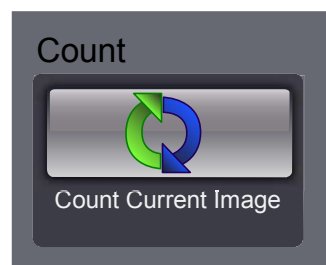
5. Click on "Preview Image for Current Assay"



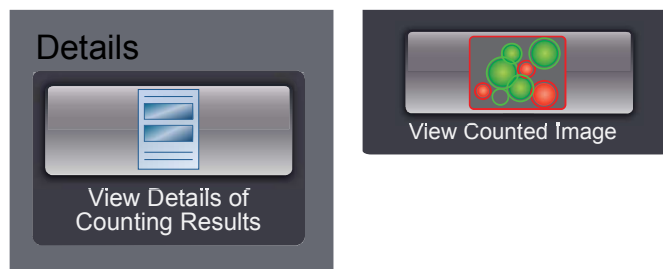
6. Adjust the focus if necessary using the coarse and fine adjustments on the left-hand side of the screen. Cells in focus will have a bright center and crisp edge.



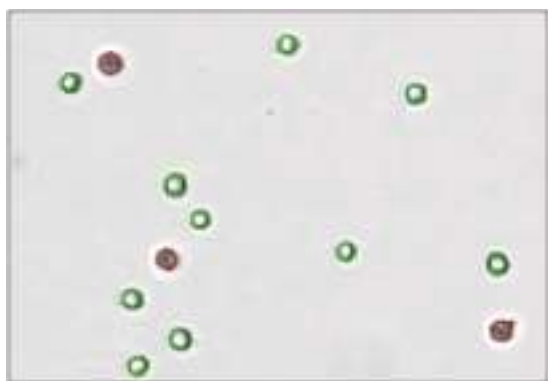
7. Click the Count button at the bottom of the screen.



8. Click on the “View Details” button at the bottom left of the screen, then click on the “View Counted Image” button on the left-hand side of the screen.



9. Review the counted image. Live cells should be circled in green. Dead cells, stained dark with trypan blue, should be circled in red.



Preparing Cell Sample for Dual-Fluorescence Viability using AO / PI

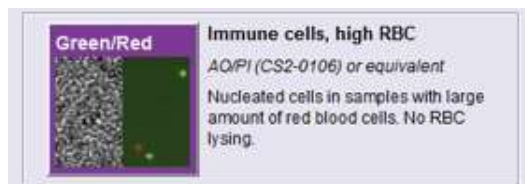
1. A cell concentration of 1×10^5 to 1×10^7 , following dilution with staining solution, is recommended for analysis on the Auto 2000. A concentration of 1.0×10^6 cells/mL is optimal.
2. Dilution or concentration of a cell sample may be required based on the initial concentration.
3. Invert the tube containing cells ten times and pipette up and down at least ten times 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.)
4. If necessary, dilute original sample with PBS. Stain cells by combining 20 μ l of cell sample with 20 μ l of ViaStain™ AO / PI staining solution. For whole blood and other viscous samples, draw sample in and out of the pipette tip prior to transfer. The table below shows the recommended dilution and final dilution value to enter into the Auto 2000 software for a variety of sample types. Gently mix by pipetting up and down ten times before adding sample to counting chamber.

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 μ l	20 μ l	20
PBMCs following Ficoll separation	Not required	20 μ l	20 μ l	2
Stem cells from CD34+ separation	Not required	20 μ l	20 μ l	2
Mononuclear cells from processed bone marrow	Not required	20 μ l	20 μ l	2
Tumor digest Tissue digest	Not required	20 μ l	20 μ l	2

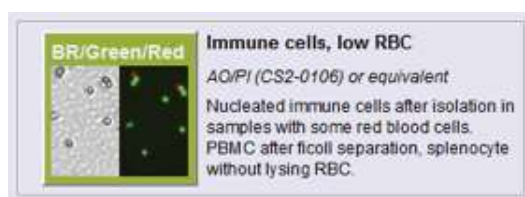
Dual-Fluorescence Viability Using AO/ PI and the Auto 2000

1. Pipette up and down gently 10x to break any potential cell clumps in the stained cell sample
2. Load 20µl of sample into the Cellometer Counting Chamber (CHT4)
3. Insert counting chamber into instrument.
4. Click on the appropriate assay, according to the primary cell type being analyzed. When prompted, enter your desired Sample ID and the dilution factor from the table in step H (accounting for any additional sample dilutions made). Each assay below is pre-optimized with specific counting parameters for the cell type(s) indicated.

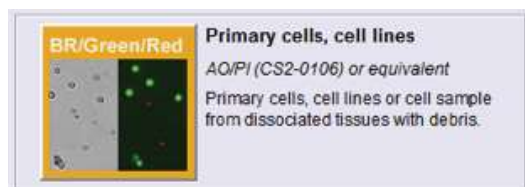
Total Nucleated Cells, or
white blood cells, from
diluted peripheral blood or
cord blood



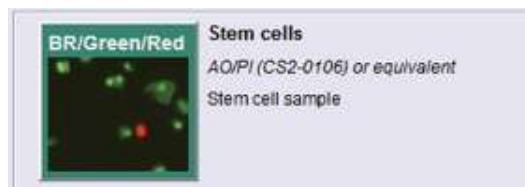
PBMCs, Splenocytes, and
other primary cells following
initial separation



Tumor Digests and **Tissue Digests**



Stem Cells



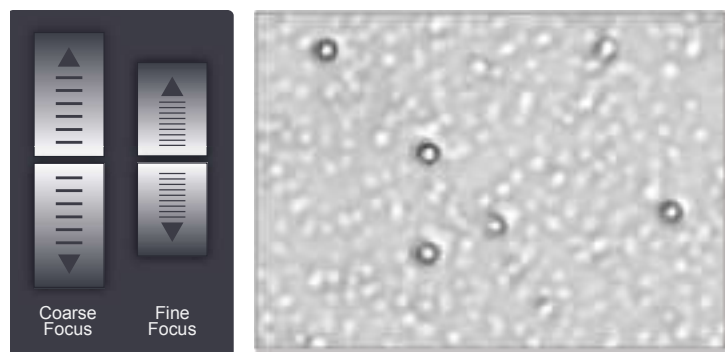
5. Click on "Preview Image for Current Assay"



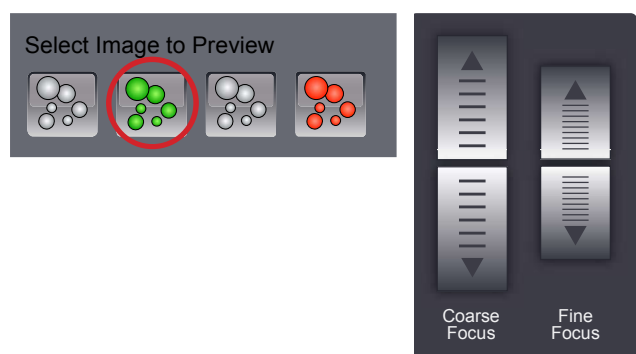
6. Select the bright field image



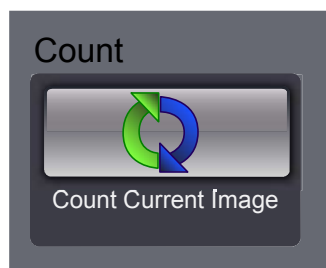
7. Adjust the focus if necessary using the coarse and fine adjustments on the left-hand side of the screen. Cells in focus will have a bright center and crisp edge.



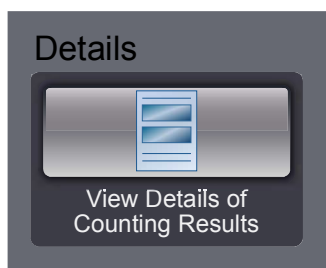
8. Confirm the focus in the green fluorescent channel. Select the green fluorescent image. The fluorescent spots should have sharp edges. If the edges appear fuzzy, the focus should be adjusted.



9. Click the count button at the bottom of the screen.



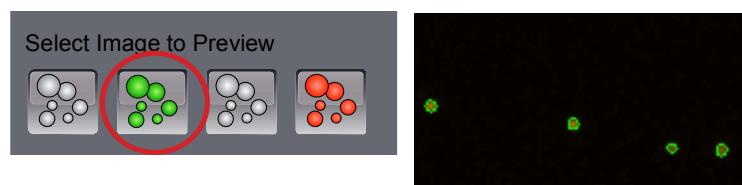
10. Click on the "View Details" button at the bottom left of the screen.



11. Select the green fluorescent image, then click on the "View Counted Image" button on the left-hand side of the screen. Enlarge the image by clicking the "Zoom In" button on the right-hand side of the screen. Confirm that all of the live (green fluorescent) cells are circled in green.



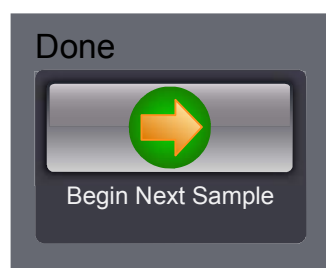
12. Select the red fluorescent image. Confirm that all of the dead (red fluorescent) cells are circled in green.



13. Click on the Return or "Back to Results" button. Cell count, concentration, mean cell diameter, and % viability are displayed.

Assay: Immune cells, low RBC		
Sample ID: PBMC_AOPI_4.2		
Dilution Factor: 2.00		
Count	Concentration	Mean Diameter
Total: 1428 cells	4.93x10 ⁶ cells/mL	6.3 microns
Live: 996 cells	3.44x10 ⁶ cells/mL	6.4 microns
Dead: 432 cells	1.49x10 ⁶ cells/mL	5.9 microns
Viability: 69.8%		

14. The Auto 2000 is now ready to analyze the next sample. After inserting the imaging chamber loaded with the next sample, click on the "Next Sample" button at the bottom right of the screen. When prompted, enter the Sample ID, then click "Count".



Tutorials

Overview

The following tutorials are intended as a guide to performing various cell counting assays using the Auto 2000. General sample preparation hints are included for each tutorial as well as instrument and software operation instructions.

Each of the assays can also be performed using the sample images included in the software as a demonstration of the Auto 2000. Sample images for each cell counting assay can be found at C:\Program Files\Nexcelom\Assay_Images\

Staining Solutions

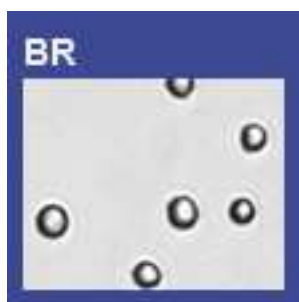
Trypan Blue Stock solution: 0.2% in PBS. Use 1:1 with cell sample. Dilution factor: **2**

AO (CS1-0108) Stock solution: 10ug/mL in PBS. Use 1:1 with cell sample. Dilution factor: **2**

PI (CS1-0109) Stock solution: 100ug/mL in PBS. Use 1:1 with cell sample. Dilution factor: **2**

AO/PI (CS2-0160) Stock solution: 5ug/mL AO; 100ug/mL PI in PBS. Use 1:1 with cell sample. Dilution factor: **2**

Installed Assays and Descriptions



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 cell 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 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 and compute the percent viability.

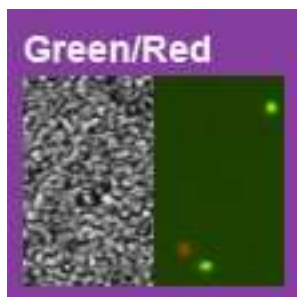


Cell Line, Viability Trypan Blue

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 and leaves live cells unstained. After taking brightfield images of the stained sample, live and dead cells are counted to determine total, live and dead cell concentrations as well as compute percent viability.



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 is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide 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 and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

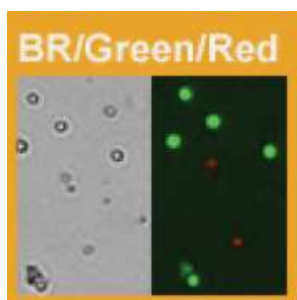


Immune Cells, Low RBC

AO/PI (CS2-0106) or equivalent

Nucleated immune cells after isolation in samples with some red blood cells. PBMC Cafter ficoll separation, splenocyte without lysing RBC.

Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide 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 and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.



Primary Cells, Cell Lines

AO/PI (CS2-0106) or equivalent

Primary cells, cell lines or cell sample from dissociated tissue with debris

Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells.

Propidium iodide 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 and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.



Stem Cells

AO/PI (CS2-0106) or equivalent

Stem cell sample

Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide 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 and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

Operation Reference

Counting Options



1. **Count All** Select to have all 4 positions of slide used for cell images
2. **Speed Count** Select to have counting use Cell Limit or Image Limit
 - a. **Use Cells Limit** Check to stop counting after # of user defined cells are counted and the next frame has finished counting
 - b. **Use Images Limit** Stop counting after finishing user defined images that are less than 4 images
3. **Done: Save Settings** Saves the current settings and returns to previous screen.

Saving Options

The screenshot shows a 'Save Options' dialog box with the following elements:

- Save Options Section:**
 - 1. Set Sample ID as Cell Type (Off)
 - 2. Auto Increment Sample ID (Off)
 - 3. Log User Name (Off)
 - 4. Time Stamp Sample ID (Off)
 - 5. Include Instrument ID in File (Off)
- Auto Save Section:**
 - 6. File: P:\Common Folders\Users\Tim\data.txt
 - 7. Auto Save to Data File (Off)
 - 8. Create New File for Each Sample (Off)
 - 9. Folder: P:\Common Folders\Users\Tim\
 - 10. Save Raw Images (Off)
 - 11. Save Counted Images (Off)
- Auto Print Section:**
 - 12. Auto Print Count Results (Off)
 - 13. Print with Default Printer (Off)
- Done Section:**
 - 14. Save Settings (Green arrow button)

Save Options

- 1. Set Sample ID as Cell Type** Selecting this will auto input the Sample ID to match the Cell Type parameter name being used for counting
- 2. Auto Increment Sample ID** Selecting this will auto append the Sample ID with an incremental numerical value (Example: CHO sample_001)
- 3. Log User Name** Selecting this will require the user to enter in an ID that will be recorded with the data
- 4. Time Stamp Sample ID** Selecting this will auto append the Sample ID with the date and time the count was performed
- 5. Include Instrument ID in File** Selecting this will auto append the Instrument ID to the sample ID after the count is performed

Auto Save

- 6. File** this button brings up the File Dialog Window which allows the user to specify where the data.txt file will be saved when Auto Save to Data File is selected.
- 7. Auto Save to Data File** Selecting this will auto save the data into the Data.txt file after a count is performed
- 8. Create New File for Each Sample** Selecting this will auto create a new data.txt file for each sample and save the counting results to it after a count is performed

- 9. Folder** this button brings up the File Dialog Window which allows the user to specify where the image folder will be saved when Save Raw Images or Save Counted Images is selected.
- 10. Save Raw Images** Selecting this will auto save the images to the folder specified using the Folder command
- 11. Save Counted Images** Selecting this will auto save the counted images to the folder specified using the Folder command

Auto Print

- 12. Auto Print Results** Select to have the results sent to the printer upon completion of count.
- 13. Print with Default Printer** Select to have the Windows default selected printer used when Auto Print Results is selected.
- 14. Done** Save Settings Click to save new settings and return to Assay and Settings main screen.

Technical Information

Specifications

Size and weight	Height 13.4 inches (340 mm) Width 11.1 inches (283 mm) Depth 12.8 inches (324 mm) Weight 26.0 Pounds (11.8 kg)
Environmental Requirements	Typical biology lab environment
Display	10.4 inch, 1024 X 768 TFT with LED back light
Touch screen	4-Pin resistive with controller 10.4 inch format
Processor	Intel® Core i3
Memory	RAM Memory: 4GB DDR2 Hard drive size: 16 or 32 GB Mini PCI3 BIOS: 2 MB flash
Operating system	Window 7 embedded system
External Connectors	2 USB 2.0 1 Ethernet 1 Power input plug
Power input	12 VDC @ 7.5 Amps
External button	On/off 2-color button Orange: Standby Blue: Run
Optics	Excitation Channel #1: 470 nm Channel #2: 540 nm Emission Channel #1: 535 nm Channel #2: 605 nm Objective: 4X plan achromat
Camera	½ inch interline 1.4 MP monochrome Pixel size: 4.65 µM square
Circulation and Cooling	Allow at least 6" of unobstructed air circulation around the vents holes on the Auto 2000 while in use to ensure adequate cooling of the product.

Getting Support

We provide free consulting for your lab on cell type, assay type and sample conditions. The goal is to find the most suitable cell counting / analysis solution for your lab.

Our team of Application Specialists are trained biologists with comprehensive understandings of cell counting, viability assay methods and best practices.

Please contact us when you need to discuss specific types of cells, sample conditions, and applications. We are available between 8:30am and 5:00pm Eastern US time. For immediate help, please call us at 1-978-327-5340 or email

support@nexcelom.com for applications and other technical information

sales@nexcelom.com for placing a purchase order or inquiries on a purchase order

info@nexcelom.com for general inquiries

Warranty Information

Nexcelom warrants that Nexcelom instrumentation products shall, for a period of 12 (twelve) months from the date of purchase, be free of any defect in material and workmanship. The sole obligation of this warranty shall be to either repair or replace at our expense the product, at manufacturers option. The original sales receipt must be supplied for warranty repair. Products, which have been subjected to abuse, misuse, vandalism, accident, alteration, neglect, unauthorized repair or improper installation, will not be covered by warranty.

Any Product being returned is to be properly disinfected and packaged (in original packing if possible). Damage sustained in shipping due to improper packing will not be covered by warranty. A valid Return Material Authorization Number (RMA#) is required for all warranty repairs. For RMA instructions, please contact our customer service department at 978-327-5340 or email support@nexcelom.com.

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Nexcelom Bioscience LLC
360 Merrimack Street
Building 9
Lawrence, MA 01843, USA
Phone: +1. 978.327.5340
Fax: +1.978.327.5341
Email: support@nexcelom.com
www.nexcelom.com

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STCL-EQUIP-022 JA1 Cellometer Auto 2000 Users Manual JA1**Author**

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		01 Feb 2021, 05:54:37 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		01 Feb 2021, 05:54:52 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		01 Feb 2021, 06:01:04 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Isabel Storch De Gracia (IMS19)		17 Feb 2021, 09:33:04 PM	Approved

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