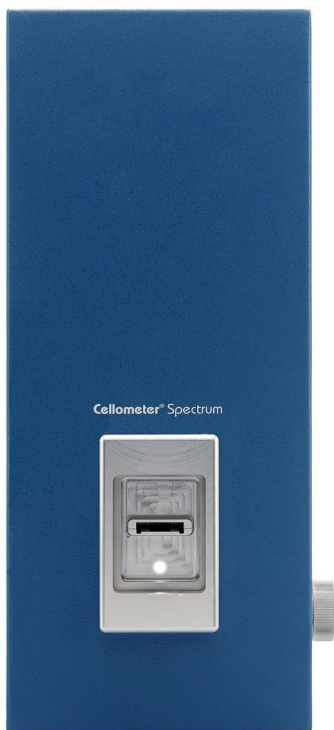


QUICK START GUIDE

CELLOMETER[®] SPECTRUM



8002638 Rev B

For Research Use Only. Not for use in
diagnostic procedures.



Cellometer® Spectrum Quick Start Guide

8002638 Rev B

February 2024

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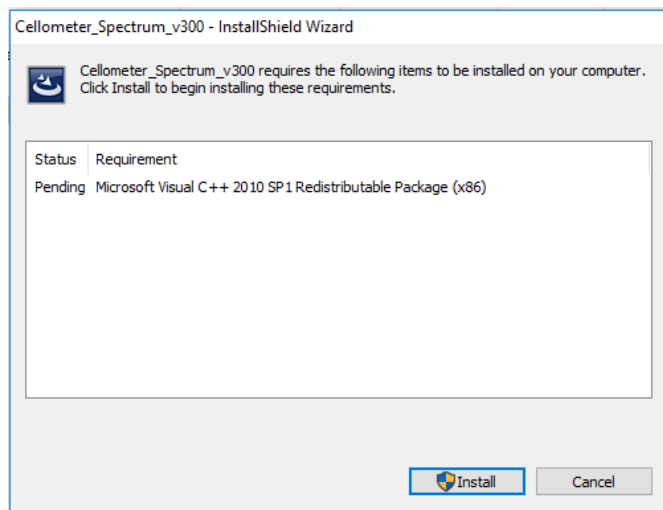
Installing the Cellometer Spectrum

Unpack Checklist

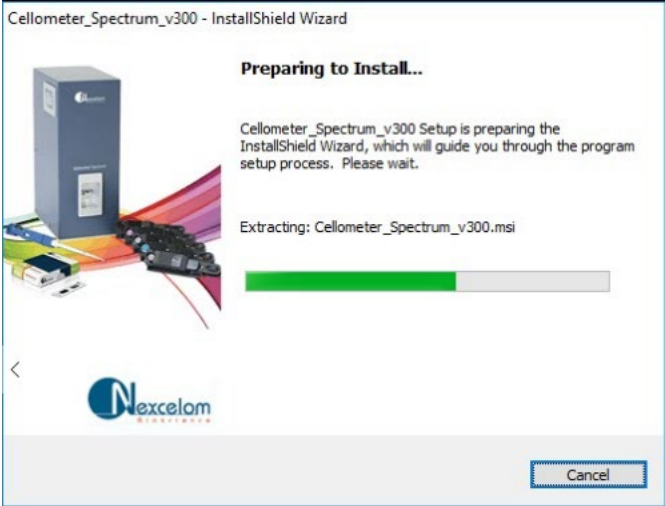
- Cellometer Spectrum Instrument
- USB 2.0 Cable
- Power Supply & Power Cord
- Cellometer Spectrum Focus Guide
- Cellometer Spectrum Software USB
- Cellometer Spectrum User Manual
- Cellometer Spectrum Quick Start Guide
- Cellometer Spectrum Simple Setup Guide
- Cellometer Fluorescence Beads Starter Pack
- Thank You Letter
- CHT4 Counting Chambers (Shipped Separately)

Software Installation

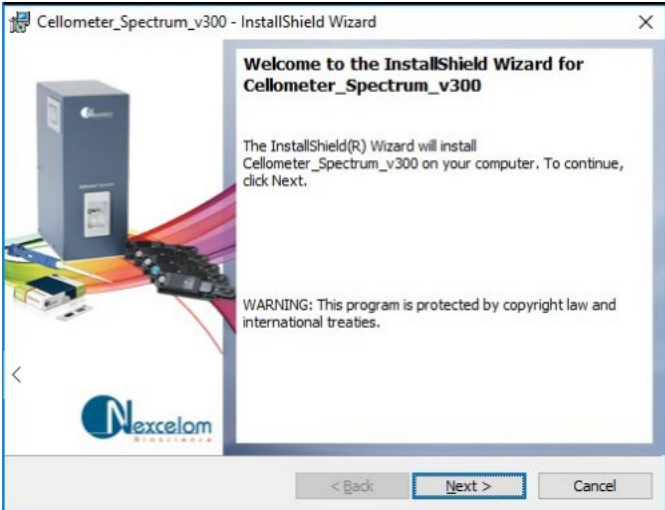
1. Installation supported in Windows 10.
2. Insert Cellometer Spectrum software USB into USB Port. Installer will auto run.
3. Open USB drive.
4. Double click on the Cellometer Spectrum software folder: **Cellometer_Spectrum_vX.X.X.X**
5. Double click on the **Cellometer_Spectrum_vX.X.X.X.exe** file.
6. Click **“Install”** (may see this step).



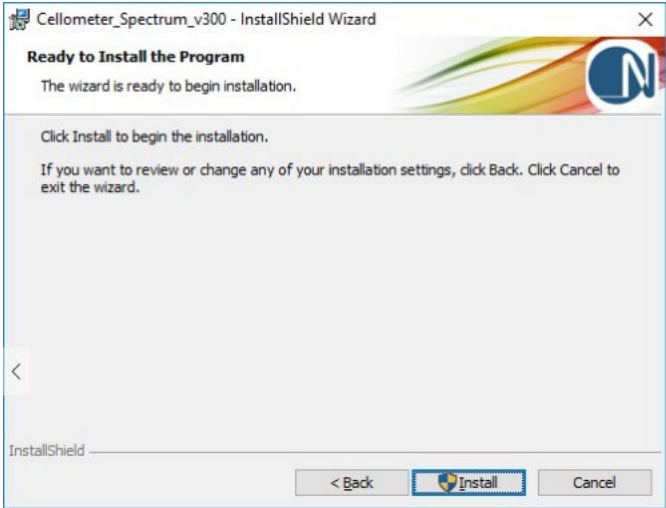
7. Installation will continue.



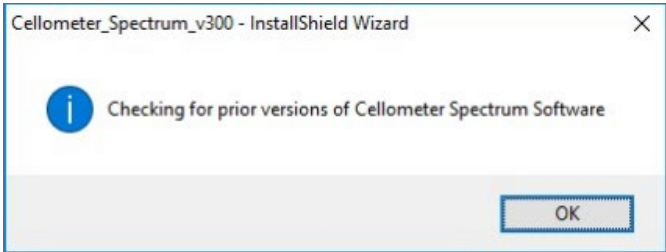
8. Click "Next >".



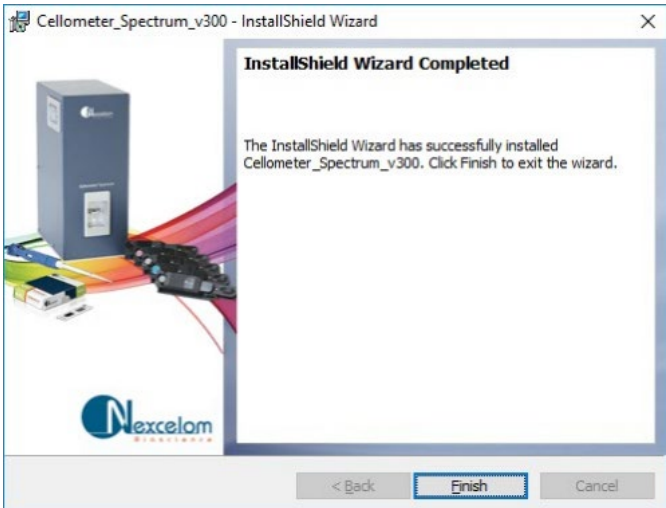
9. Click **“Install”**.



10. Click **“OK”**.



11. Click **“Finish”**.



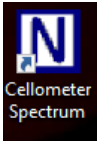


- 12. The Cellometer Spectrum icon will appear on your desktop.
- 13. Close the **Cellometer_Spectrum_vX.X.X.X** folder.
- 14. Remove the USB drive from the USB port.

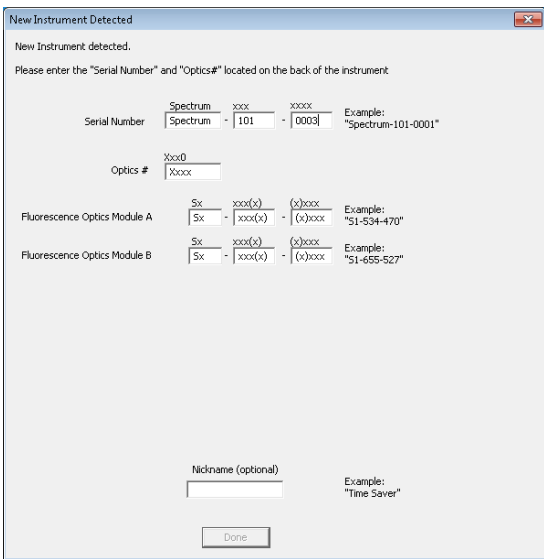
USB Driver Software Installation

- 1. Connect power to Cellometer Spectrum instrument located on the back of the unit.
- 2. Connect the USB 2.0 cable between Cellometer Spectrum instrument and a USB 2.0 port on the computer. (Make sure that Cellometer Spectrum software installation is complete before connecting the USB 2.0 cable.)
- 3. Windows OS will automatically install the USB drivers (This may take up to several minutes).

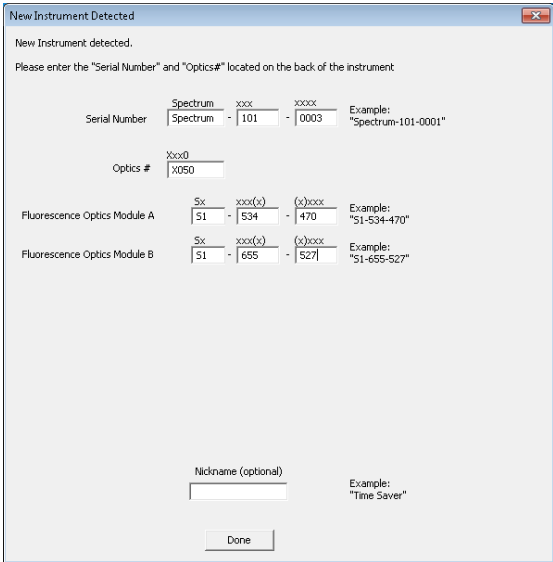
Starting the Cellometer Spectrum



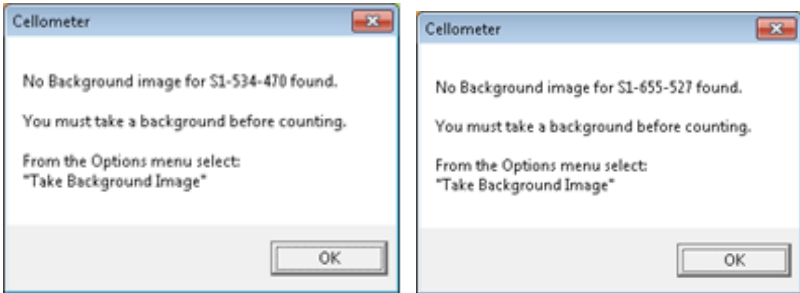
- 1. Start Cellometer Spectrum software by clicking the icon on your desktop.
- 2. Enter instrument serial number which is located on the back of the Cellometer Spectrum.



- 3. Enter in Optics # and Fluorescence Optics Module A and Fluorescence Optics Module B information.

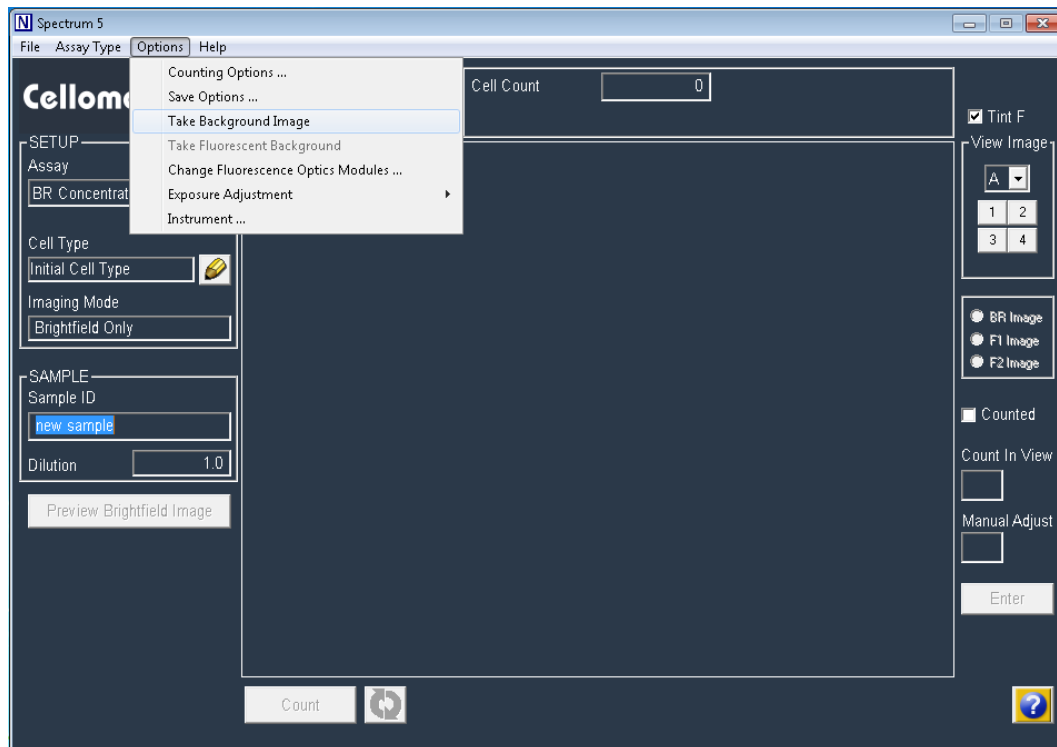


- 4. Click "Done".
- 5. You will see two warnings that a background image must be taken if this is the first time the instrument has been connected to the computer. Click "OK" for both.

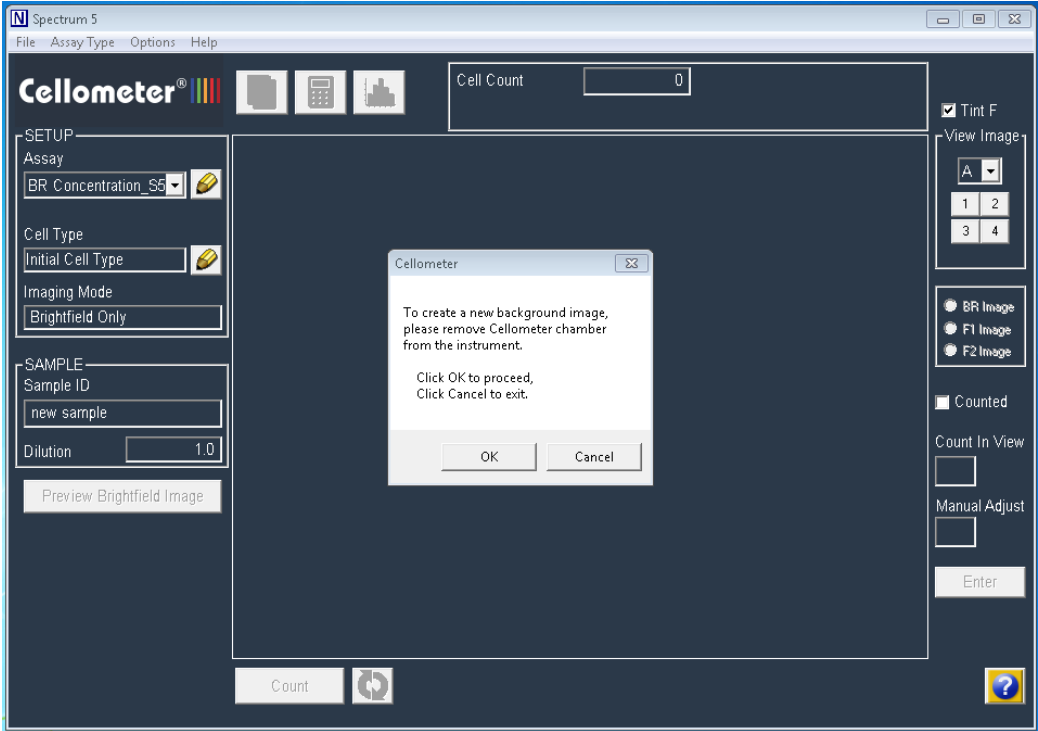


Taking a Background Image

1. When using the Cellometer Spectrum for the first time, a background image should be taken. This step does not need to be repeated unless the instrument is moved.
2. Click on menu item “Options”, then “Take Background Image.”

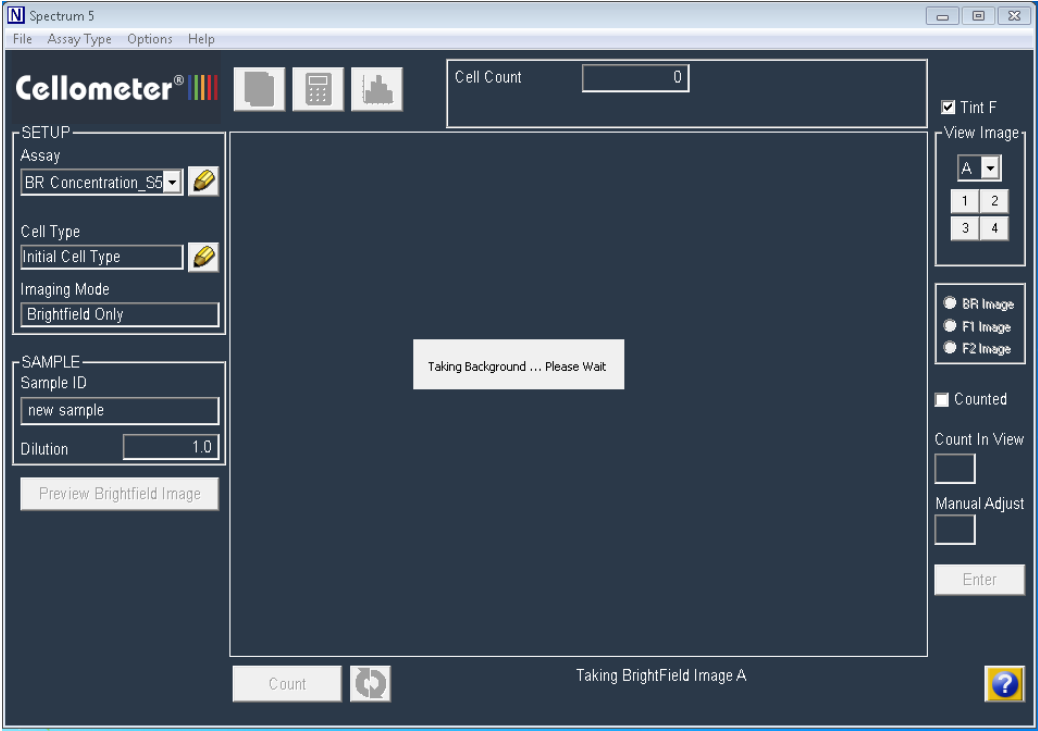


3. Confirm that there is no slide in the Cellometer Spectrum instrument.

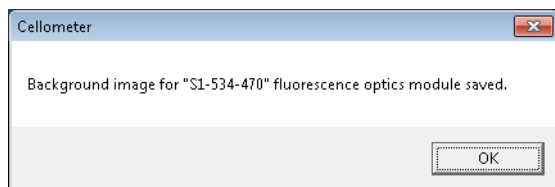


4. Click "OK".

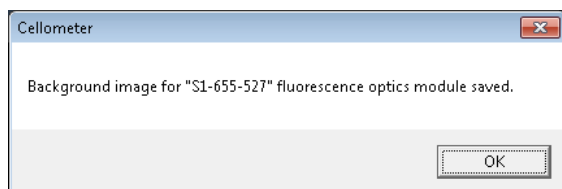
5. Cellometer Spectrum software will now take a new Background Image for Optics Module A.



6. When done click "OK".



7. Cellometer Spectrum software will now take a new Background Image for Optics Module B.
8. When done click "OK".



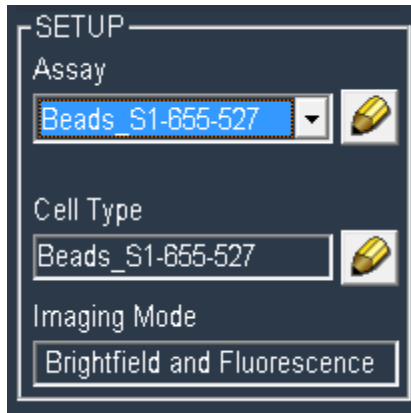
Preparing Reference Beads

1. Though the Cellometer Spectrum instrument does not require any routine testing or calibration, reference beads are available.
2. Cellometer Check Reference Bead Mixture, product number CCBM-011-2ML, is recommended for use with the Cellometer Spectrum.
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.

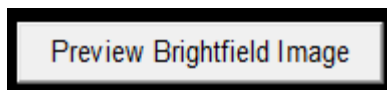
Counting Reference Beads

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 Cellometer Spectrum sample slot and gently push the slide to the stop.

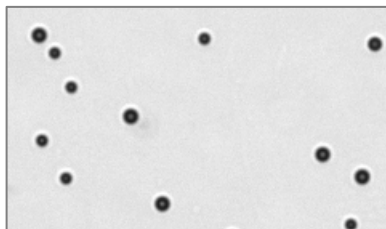
5. Select the Beads_S1-655-527 assay.



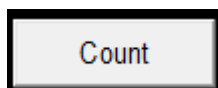
6. Click on "Preview Brightfield Image".



7. Adjust the focus if necessary until the best bead counting focus is achieved. The beads should appear as dark circles with sharp edges.

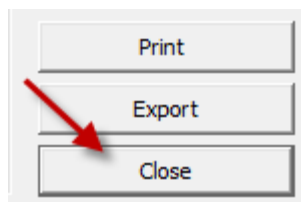


8. Click the **Count** button at the bottom of the screen.

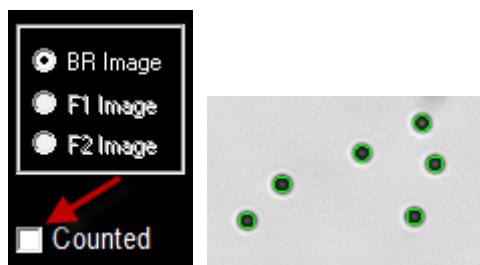


9. When counting is complete the result screen will appear with bead *Count*, *Concentration* and *Mean Diameter*.

10. Click on “Close” to return to main screen.



11. Click on “Counted” button to view which beads have been counted (indicated by green outlines).



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 Spectrum prior to staining.

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 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. There is no interference from debris and non-nucleated cells.

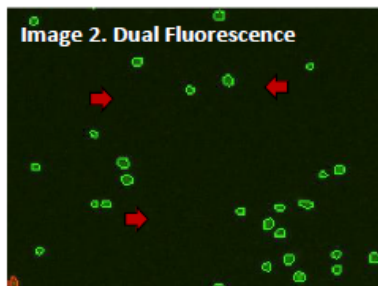
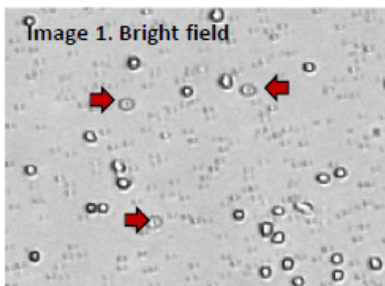


Image 1 and 2. Red blood cells seen in the bright field image are not present in the fluorescent image that is used by Cellometer for AO/PI cell counting. Only nucleated cells are counted, resulting in a more accurate total cell count and % viability calculation.

Preparing Cell Sample for AO/PI Viability Determination

1. A cell concentration of 1.0×10^5 to 1.0×10^7 cells/mL can be analyzed on the Cellometer Spectrum. 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 20 μ L of cell sample with 20 μ L of AO/PI staining solution (CS2-0106-5ML). Gently mix by pipetting up and down ten times.

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:

- **8002632 Cellometer Spectrum User Manual** for instrument operation, care and maintenance details (available as a PDF file on the USB Drive)
- **8002633 Cellometer Spectrum 5X Focus Guide** or **8002634 Cellometer Spectrum 10X Focus Guide** for assistance with optimal focus