Introduction

Viability is a measure of the metabolic state of a cell population that indicates the potential for growth. Trypan blue viability is a dye exclusion method that utilizes membrane integrity to identify dead cells. Trypan blue dye is unable to penetrate healthy cells, so they remain unstained. Dead cells have a compromised cell membrane that is permeable to the trypan blue dye. Dead cells are stained blue and display as dark cells that can be identified by the Cellometer software with bright field imaging.

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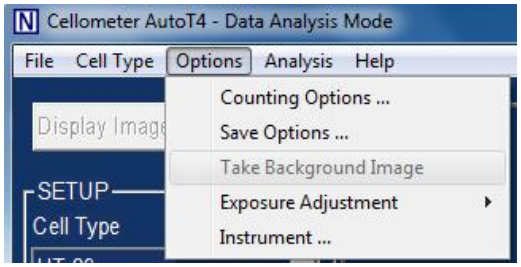
A. Preparing the Cellometer Auto T4

1. Turn on power switch.
2. Double click the Cellometer icon 

on the desktop to start the Cellometer Software

3. If using the Cellometer Auto T4 for the first time, take a background image. From the **Options** menu, select "**Take Background Image**".



4. After the background image has been taken click the **Display Image** button at the top left of the screen. Verify that a uniform gray image is displayed and click **OK** to proceed.

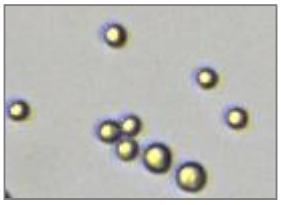
B. Preparing Your Cell Sample

1. A cell concentration of 2.5 x 10^5 to 1.0 x 10^7 cells/mL is recommended
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. If possible, avoid shaking or vortexing the sample, as this may generate bubbles that make it difficult to pipette accurately.

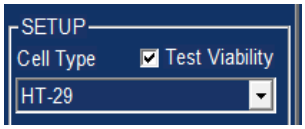
3. For viability measurement, stain cells by combining 50 µl of cell sample with 50 µl of a 0.2% trypan blue staining solution (for a final concentration of **0.1% trypan blue**). Gently mix by pipetting up and down ten times.

C. Loading a Sample

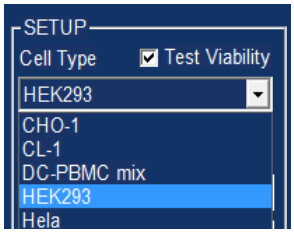
1. Peel plastic off of BOTH sides of the Cellometer slide. (for PD100 slides, the plastic has already been removed)
2. Place cell counting chamber on a fresh Kimwipe
3. Label slide chamber #1 and chamber #2 on the white margin of the chamber. Avoid touching the clear portion of the counting chamber.
4. Load 20 µl of mixed cell sample into the Cellometer counting chamber.
5. Insert the loaded chamber into the Auto T4 sample slot and gently push the slide to the stop.
6. Click the **Display Image** button at the top left of the screen.
7. Slowly turn the "**Focus Knob**" until the best cell counting focus is achieved. Live cells have a bright center and a clearly defined edge.



8. Check the **Test Viability** box.

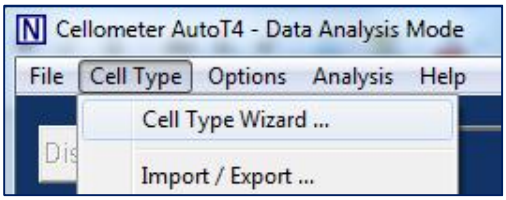


9. Select the Cell Type from the drop-down menu or create a new cell type (see section D below) then proceed to section E.

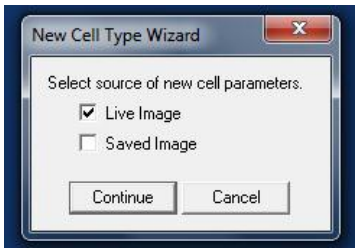


D. Creating a New Cell Type

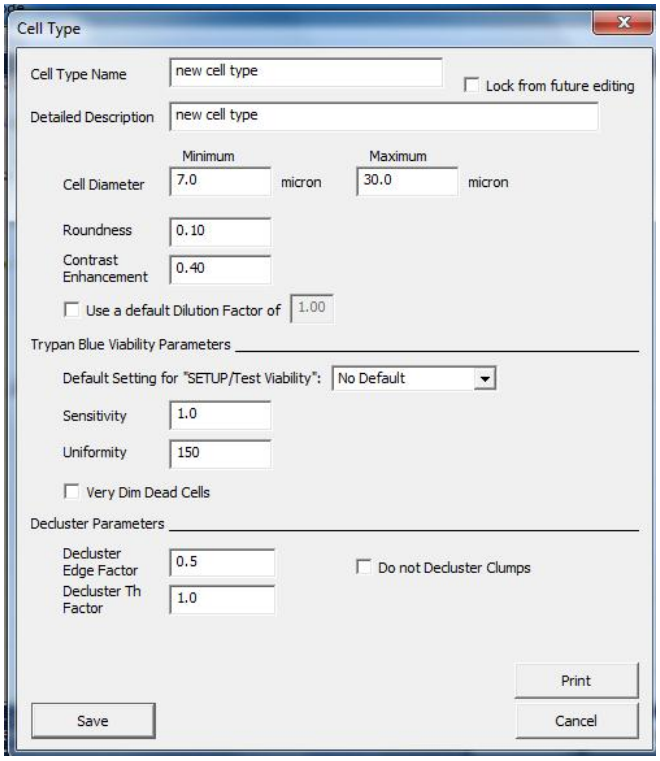
1. Select the **Cell Type Wizard** from the **Cell Type** drop-down menu to create a new cell type.



2. Check **Live Image** and click **Continue**



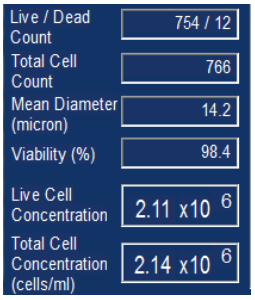
3. Enter a new **Cell Type Name** and **Detailed Description** (optional) and click **Save**.



E. Analyzing a Sample

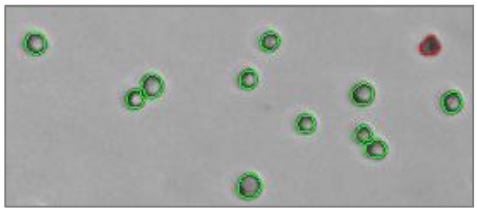
1. After selecting or saving the cell type, enter the **Sample ID** and **Dilution factor**. For undiluted samples (cell counting only), the Dilution factor is 1. If cells were stained with trypan blue at the recommended 1:1 dilution for viability determination, the Dilution factor is 2.

2. Click the **Count** button at the top left of the screen. When counting is complete, the software will display the Live / Dead Cell Count, Total Cell Count, Mean Diameter, % Viability, Live Cell Concentration, and Total Cell Concentration in the table at the bottom left of the screen.



3. If the Total Cell Count is < 100, the cell sample is too dilute. Analyze a more concentrated sample using the appropriate cell type from the drop-down menu. If the Total Cell Count is > 3,000, dilute the original cell sample in the appropriate media or PBS and analyze using the appropriate cell type from the drop-down menu.
4. Check the **Counted** box and click 1 (for image 1 of 4) in the View Image section at the right hand side of the screen.

5. Live cells with a bright center should be circled in green. Dead cells which are dark should be circled in red (if trypan blue viability is being tested). Individual cells within clumps or doublets should be circled.



F. Technical Support

If it appears that live cells, dead cells and/or cell clumps are not being analyzed correctly or debris is being counted, please contact Nexcelom Technical Support at support@nexcelom.com or 978-327-5340. A Nexcelom Technical Support Specialist is available from 9am to 5pm EST Monday through Friday for assistance with optimization of cell type parameter settings.