Cellometer Auto T4 Quick Start Guide

Contents of Shipping Container

- ☐ **Cellometer Auto T4 Instrument**

- ☐ **Power Cord**

- ☐ **USB Connector Cable**

- ☐ **USB Drive** – Contains **Cellometer Auto T4 Software**

- ☐ **Cellometer Auto T4 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

- ☐ **Revvity-provided Laptop** (optional)

1. **Display Image Button** – Shows cells currently in the counting chamber.
2. **Count Button** – Initiates counting procedure.
3. **Test Viability** – Indicates that a viability stain has been used.
4. **Cell Type** – Identifies cell type parameters to be used when counting.
5. **Sample ID** – Allows entry of a unique sample identifier.
6. **Dilution Factor** – Allows entry of final dilution factor to calculate concentration.
7. **Live/Dead Count** – Displays live and dead cells found if *Test Viability* is selected.
8. **Cell Count** or **Total Cell Count** – Displays total cells counted in the images taken.
9. **Mean Diameter (micron)** – Identifies mean diameter of counted cells in microns.
10. **Viability (%)** – Identifies percent viability of cell sample.
11. **Live Cell Concentration** – Indicates calculated live cell concentration.
12. **Cell Concentration** or **Total Cell Concentration (Cells/mL)** – Indicates calculated cell concentration/total cell count concentration.
13. **Recount Icon** – Re-analyzes images for current scan and performs a new count.
14. **Select Data File Icon** – Opens Windows File Explorer for saving count results.

15. **Image Area** – Displays images captured by the instrument.
16. **Save Data Button** – Enables users to save the data in the indicated file.
17. **View Saved Data Button** – Displays the data that would be saved.
18. **Calculate Concentration Adjustment Icon** – Activates *Sample Adjustment* tool.
19. **Cell Size Analysis Icon** – Activates *Cell Size Analysis* tool generating a histogram of cell size data to assist users with optimization of cell diameter parameters.
20. **Print Results Icon** – Prints results to selected printer.
21. **Export to Excel Icon** – Exports results to MS Excel worksheet.
22. **View Image** – Toggles between images *A* and *B*; zooms into fields of view *1-4*.
23. **Counted** – Displays graphic overlay highlighting counted cells.
24. **Count in View** or **Live/Dead Count in View** – Displays total number of cells in the currently selected view if *Test Viability* is selected.
25. **Manual Adjust** or **Live/Dead Manual Adjust** – Enables users to manually adjust cell numbers by manually entering a count of cells in view.
26. **Help Icon** – Provides users with access to online help contents and resources.

Unpacking the Instrument

Unpack and visually inspect the Auto T4 to ensure no physical damage has occurred during shipping. For assistance in setting up the instrument, visit the Cellometer Auto T4 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 T4.

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.

Operating Computer Requirements

If you did *not* purchase an Operating Computer from Revvity to run your Auto T4 instrument, the following minimum specifications are required:

- Windows 7, 8 or 10 PC
- 2.2 GHz or higher processor
- 4 GB RAM
- USB 2.0 port or higher
- Display resolution: 1024 x 768 pixels or higher

Installing the Software

The *Auto T4 System* is comprised of the instrument connected via USB cable to an Operating Computer used to run the instrument software. The Operating Computer can be linked to a network for accessing external files, printers and for storing count results.

*If the Operating Computer was purchased from Revvity, software will be pre-installed and you can skip this section. Continue to **Starting the Auto T4**.*

Ensure that instrument is *not* connected to the computer during software installation until specified in the procedure.

1. Insert Software USB Drive that was included with the instrument into USB port of the Operating Computer.
2. Extract files contained on the USB to the computer desktop.
3. On the desktop of Operating Computer, double-click the **Cellometer_version**.**exe** file (where **version** represents version being installed).

4. Click **Next** to begin the Auto T4 software installation.
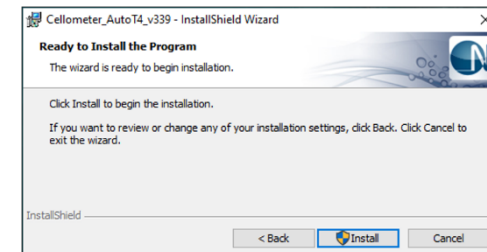
5. Click **Install** to install the instrument software on the hard drive of the Operating Computer. *A screen containing prerequisite software to be installed on your computer may be displayed. Click **Install** to continue.*
6. When prompted, click **OK** to check for prior versions of the software.
7. Click **Finish** to exit the installer and confirm that **Cellometer Auto** software icon now appears on desktop of Operating Computer.
8. Remove the Software USB and store it per company guidelines.
9. Plug power cord for the instrument into an outlet and power ON.
10. Once software installation is complete, connect USB 2.0 cable between the Auto T4 and a USB 2.0 port on the Operating Computer.
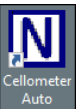
*Windows automatically installs necessary USB drivers on to the instrument which may take up to several minutes.*

Using Instrument for the First Time

Starting the Auto T4

1. On the desktop of Operating Computer, launch the Auto T4 software by double-clicking the **Cellometer Auto** software icon.

2. Enter instrument Serial Number from the device label into the input fields. The device label can be found on the back of instrument.
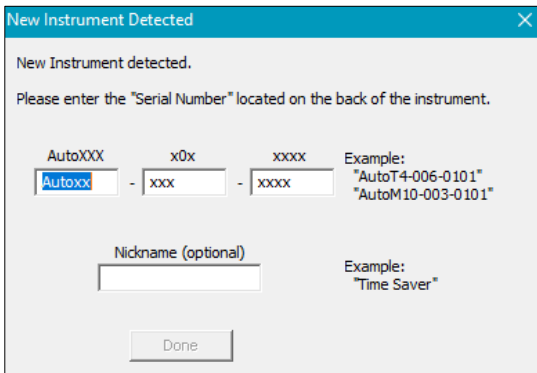
3. Click **Done** to save the instrument Serial Number.
4. A pop-up message will appear indicating a background image needs to be taken before counting. Click **OK** to continue.
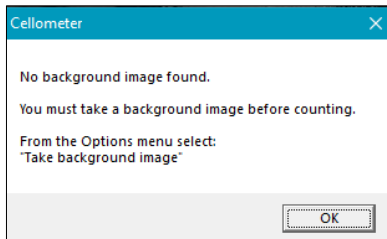
Taking a Background Image

After initial setup of the instrument, you must take a background image of the system by itself (without 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. On the desktop of Operating Computer, launch the Auto T4 software by double-clicking the **Cellometer Auto** icon.
2. Navigate to the Options Menu and select *Take Background Image* option.

3. Remove slide from instrument, if applicable. Click **OK** 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 image has been saved. Click **OK**.
5. Click the **Display Image** button located in top left corner of screen.
6. Confirm that background is a uniform gray color. If there is any discoloration (e.g., light/ dark areas), contact Support via email: CellC-support@revvity.com

Setting Up Auto Save Feature

1. Open the Options Menu and select the *Save Options…* option.
2. To automatically save data, click the **Auto Save data.txt** check box to select it. *By default, count results will be saved to a single data.txt file in which rows (logged with date/time stamp and Sample ID) are appended each time data is saved.* To change the location or name of this data file, click the **Set Folder** button, navigate to a new location, enter a file name and click **Open** to create the file.
3. To automatically save data to a new data file each time cells are counted, click the **Create new data.txt with each sample** check box to select it. *Keep in mind selecting this option may result in a large volume of files.* Click the **Set Folder** button, navigate to a new location, enter a file name and click **Open** to create the file.
4. To automatically save images, click the **Auto Save Sample Image** check box to select it. Indicate whether you want to:
   - **Save Raw Images** (saved as .png files; images can be loaded back into Auto T4 for reanalysis),
   - **Save JPG Copies** (images *cannot* be loaded back into Auto T4; use for adding to presentations or reports), or
   - **Save Counted Images** (also saved as .jpg files; images include graphic overlays of outlined cells).

   Click the **Set Folder** button, navigate to a location on the Operating Computer or network where image files can be saved, enter a file name and click **Open** to return to create the file.

Preparing Sample Slides

Although the Auto T4 does not require routine testing or calibration, counting beads are available to verify instrument functionality. *Revvity counting beads BO5-02-050 (5 μm), B10-02-020 (10 μm) and B15-02-010 (15 μm) are recommended for use with Cellometer instruments.*

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 (20x) 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 slide in the white area and insert counting chamber containing sample into *Auto Sample Slot* until touching the internal stop.
8. Click the **Display Image** button.

Note: Cell concentrations of $1.0 \times 10^5 - 1.0 \times 10^7$ cells/mL can be analyzed on the Auto T4, 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.
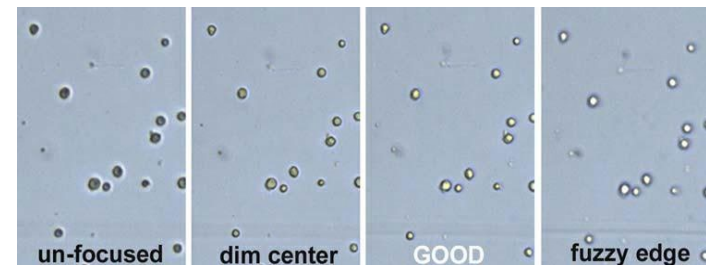
Adjusting Instrument Focus

While previewing a sample slide loaded into the instrument, adjust focus by slowly turning the Focus Knob located on the right side of the Auto T4. Proper focus is key to ensuring accurate cell counts.

Counting beads will appear as dark circles with sharp edges.
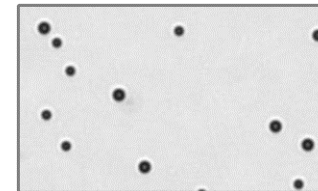
Live cells should have a bright center and dark, crisp clearly defined edges.

un-focused   dim center   GOOD   fuzzy edge

After good focus has been achieved, the instrument should perform most counting operations with only minor adjustments. You are now ready to begin automated cell counting using the Auto T4.

Counting and Analysis Workflow

Once a counting chamber loaded with a prepared sample has been inserted into the instrument, perform the following steps:

1. In the *Setup* area of the Home screen, choose a cell type from the **Cell Type** drop-down list, enter a **Sample ID** and ensure **Dilution Factor** is accurate.

2. While the cell image is displayed, click the **Count** button. *When software is in Data Analysis Mode, click the **Recount** icon* 🔄 *to re-analyze current scan and perform a new count.*

3. Once counting is complete, results are displayed in the bottom left and right corners of the screen. Use the controls in the *View Image* area to view different areas of counting chamber.

4. Confirm data is accurate by clicking the **Counted** check box to view image using a graphic overlay outlining counted cells (e.g., green for counted cells and yellow for cells *not* counted because they are larger than the specified cell diameter).

5. If testing for cell viability, select the **Test Viability** check box in the *Setup* area. Additional count results including *Viability (%)* are displayed. Click the **Counted** check box to view image using graphic overlay outlining counted cells (e.g., green for live cells, red for dead cells and yellow for cells *not* counted because they are larger than the specified cell diameter).
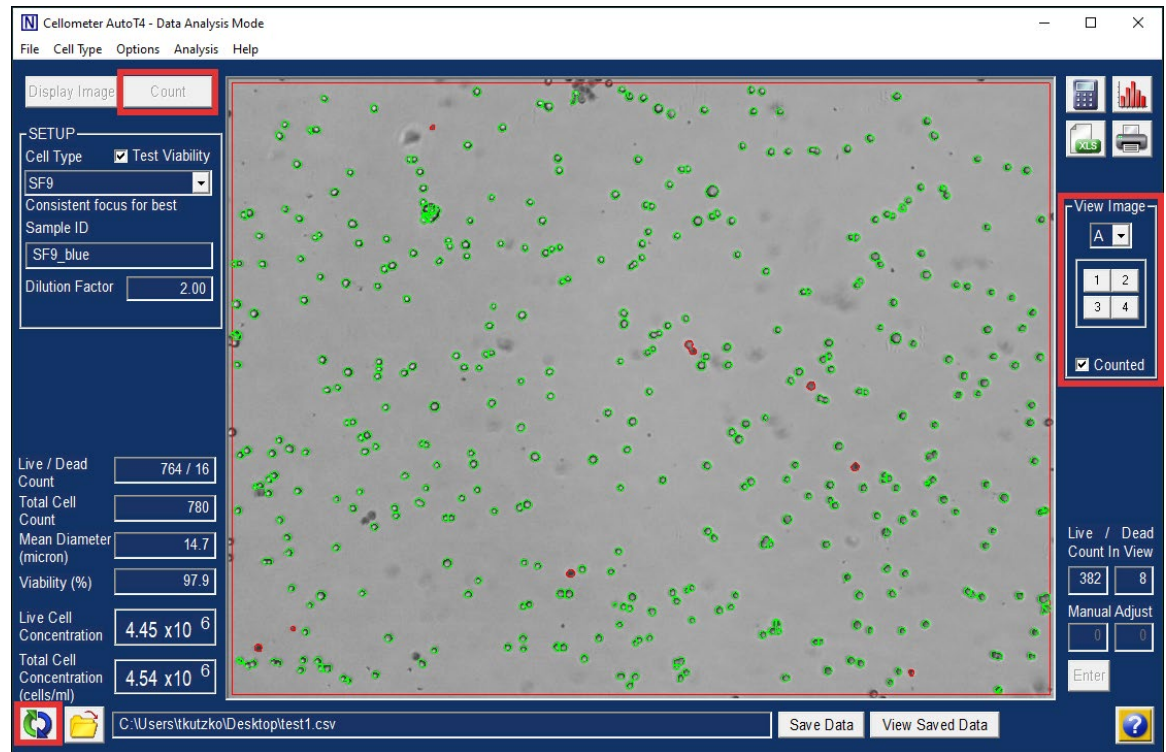
Cell Size Analysis Tool

The *Cell Size Analysis* tool 📊 displays cell size data from count results by generating 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.

Sample Adjustment Tool

The *Sample Adjustment* tool 🧮 assists you in calculating the adjustment necessary to achieve a specified target concentration or target number of cells in a tube.
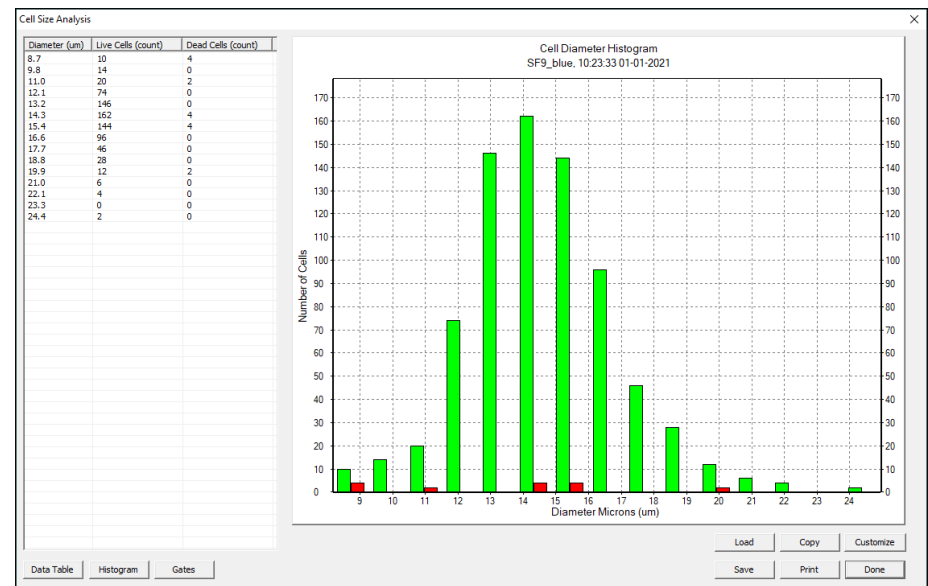
Using Trypan Blue Viability Method

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 T4 prior to staining.

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 viability is *not* recommended for samples containing debris, platelets or red blood cells. In these cases, fluorescence is required to accurately differentiate nucleated cells from platelets, red blood cells and debris.

Preparing 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 T4, 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.

Cell Concentration and Trypan Blue Viability

1. Pipette up and down gently ten times (10x) to break up any potential cell clumps in the stained cell sample.
2. Load 20 µL of sample into the Cellometer counting chamber (CHT4).
3. Hold the loaded chamber in the white area and insert counting chamber containing sample into Auto Sample Slot until touching the internal stop.
4. Click the **Display Image** button in the top left corner of screen and adjust instrument focus using knob on side of instrument.
5. Click the **Test Viability** check box located in *Setup* section of left panel.
6. Click the **Count** button to perform the count.
7. When counting is complete, results for *Live/Dead Cell Count*, *Total Cell Count*, *Mean Diameter (micron)*, *Viability (%)*, *Live Cell Concentration* and *Total Cell Concentration* are displayed in bottom left corner of screen.

Note: If *Total Cell Count* is *< 100*, the cell sample is too dilute. Analyze a more concentrated sample and be sure to select the specific cell type. If the *Total Cell Count* is *> 3,000*, dilute the original cell sample in the appropriate media or PBS, and perform the count again.

8. Review counted image by clicking the **Counted** check box located in the *View Image* section of right panel. A graphic overlay is displayed outlining counted live cells in green and outlining counted dead cells that have been stained dark with trypan blue in red. Individual cells within clumps or doublets should be circled.
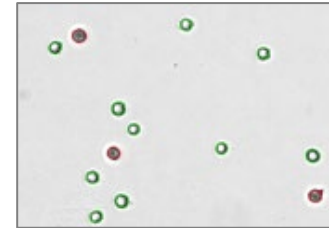
9. If Auto Save feature has *not* been enabled, save your data (if desired).

    The Auto T4 is now ready to analyze the next sample. After replacing the counting chamber previously loaded into the instrument with a new sample, click the **Display Image** icon and repeat the counting process.

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 with sample analysis and optimization of assay/cell type imaging parameters.

See the following documentation for additional instrument information:

- ***8001009 Cellometer Auto T4 User Manual*** for instrument operation, care and maintenance details (available as a PDF file on the USB Drive)

- ***8001197 Cellometer Auto T4 Focus Guide*** for assistance with optimal focus