

# Cellometer® Auto T4

## Brightfield Cell Counter



## User Manual

## **Cellometer® Auto T4 User Manual**

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# Chapter 1. Introduction

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This chapter presents introductory information to be reviewed *prior* to unpacking your Cellometer® Auto T4 instrument. It includes a product overview, lists contents of shipping container, identifies symbols/abbreviations used on both the device label and instrument, and provides a summary of topics contained in this user manual.

## AUTO T4 SYSTEM OVERVIEW

*Cellometer® Automated Cell Counters* incorporate the basic principles of Imaging Cytometry traditionally used in manual cell counting via hemocytometer into a suite of instruments designed to simplify the cell counting process. By capturing multiple images of the cells to be counted, users can adjust parameters based on cell morphology and perform reanalysis as necessary. *Cellometer Cell Counting Chambers* are equivalent to all four corner squares of a hemocytometer and are disposable, thus saving time and effort, and eliminating any risk of cross-contamination.

Using Brightfield imaging and pattern-recognition software, the Cellometer Auto T4 quickly identifies individual cells and accurately calculates *Cell Count*, *Mean Diameter (micron)* and *Cell Concentration (cells/mL)*. Customizable cell type parameters are designed to assist with the declustering of clumpy cell lines. For cultured cells stained with trypan blue, the Auto T4 simultaneously calculates *Live/Dead* and *Total* cell counts, *Viability (%)*, and *Live* and *Total* cell concentrations, including a helpful graphic overlay that may be used to highlight counted live and dead cells with color-coded outlines in the image being viewed.

The small footprint of the Auto T4 is designed to fit in small lab spaces and Cellometer Auto software can be loaded on most computers. With an Operating Computer connected to the instrument via USB cable, the system can be linked to a network for accessing external files, printers and storing count results. For laboratories that require QC access and audit controls, optional GMP/GLP software can be added to the Auto T4 for tracking audit details, offering multi-layer access control and allowing automatic printing of data/images using custom templates.

### Intended Use

Intended use of the Auto T4 is to count clean cell lines and cultured primary cells such as CHO, Jurkat, C6, HT29, DLD, KAT4, GHOST, MCF-7 and more. It can also provide total cell count and viability using trypan blue.

Personnel operating this instrument are encouraged to familiarize themselves with device controls and operation. Ensure that users can identify all components associated with the instrument, perform adequate adjustments and understand performance criteria. If issues are encountered, follow the [Troubleshooting](#) guidelines on page 57 to restore performance if instrument does not meet or exceed defined performance criteria.



**WARNING:** Use of controls or adjustments or performance of procedures other than those specified herein or by Nexcelom Bioscience LLC. may result in a hazardous process.

## CONTENTS OF SHIPPING CONTAINER

### Cellometer Auto T4 Instrument

**Power Cord** (Figure 1)

**USB Connector Cable** (Figure 2)

### USB Drive

Contains Cellometer Auto T4 Software; used to install software on an Operating Computer *not* provided by Nexcelom

**Note:** Store USB drive where it can easily be located if software needs to be installed on another computer.

### Cellometer Auto T4 User Manual and Quick Start Guide

Available as .pdf files on the USB Drive with software

### Focus Guide

Graphic sheet intended to help users adjust instrument focus

### Cell Counting Chambered Slides (Figure 3)

One box of 75 disposable slides; each slide contains two separate counting chambers

### Nexcelom-provided Laptop (Figure 4)

If a laptop was purchased from Nexcelom (optional) along with the instrument, it will be shipped in a separate box. This laptop will have the specifications listed below:

- Windows 10 OS 64 bit
- Intel Core Processor i3-8130U (2.20GHz)
- 4GB RAM
- 500GB 7200RPM SATA NoteBook Drive
- 15.6" 1366 x 768 HD LED Wide Screen

If a laptop was *not* purchased from Nexcelom, the following minimum specifications are required for an Operating Computer:

- Windows 7, 8, or 10 PC
- 1.6 GHz or higher processor
- 2GB RAM (4GB RAM or higher recommended)
- USB 2.0 or higher
- Display resolution: 1024 x 768 pixels or higher

**Note:** An Intel Mac with Parallels/Windows may be substituted for a Windows computer, but this setup is *not* recommended.

Figure 1. Power Cord



Figure 2. USB Connector Cable



Figure 3. Chambered Slide

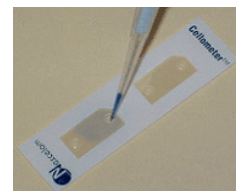


Figure 4. Nexcelom Laptop






## ABOUT THIS USER MANUAL

This *Cellometer Auto T4 User Manual* provides information on the following topics:

- System Overview and Shipping Contents
- Potential Hazards and Safety Information
- Components, SN Label and System Specs
- Site Preparation and Unpacking
- Software Installation and First-time Use
- Key Software Screens and Menus
- Slide Preparation and Loading Samples
- Basic Counting and Analysis Workflow
- Cell Types and Adjustment Parameters
- Saving/Exporting Data and Image Files
- Cleaning, Maintenance and Storage
- Troubleshooting and FAQs
- Nexcelom Support Services and Contacts
- Consumables, Conformity and Warranty

The following *Precaution Signifiers* are used in conjunction with the  symbol in this user manual:



**IMPORTANT:** Note indicating that to skip or move past *<content\_of\_note>* may result in improper functionality of the instrument.



**CAUTION:** Note indicating that *<content\_of\_note>* may damage instrument to the point where it will no longer function as expected.



**WARNING:** Note indicating that *<content\_of\_note>* may permanently damage instrument and cause personal injury or harm.














## GLOSSARY OF ABBREVIATIONS

The following abbreviations may be displayed on the shipping container, on the Cellometer Auto T4 device label or in this user manual.

<b>A</b>	Amperes	<b>kW</b>	Kilowatt
<b>AC</b>	Alternating Current	<b>lbs</b>	Pounds
<b>ANSI</b>	American National Standards Institute	<b>PC</b>	Personal Computer
<b>AO</b>	Acridine Orange	<b>PI</b>	Propidium Iodide
<b>API</b>	Application Program Interface	<b>LED</b>	Light-emitting Diode
<b>@</b>	at	<b>MHz</b>	Megahertz
<b>BR</b>	Brightfield	<b>μL</b>	Microliter
<b>°C</b>	Degrees Celsius	<b>μm</b>	Micron
<b>Cm</b>	Centimeter	<b>mL</b>	Milliliter
<b>EU</b>	European Union	<b>mm</b>	Millimeter
<b>FAS</b>	Field Application Scientist	<b>ms</b>	Millisecond
<b>FCC</b>	Federal Communications Commission	<b>nm</b>	Nanometer
<b>FDA</b>	Food and Drug Administration	<b>OQ</b>	Operational Qualification
<b>FL</b>	Fluorescence	<b>OSHA</b>	Occupational Safety and Health Administration
<b>GUI</b>	Graphical User Interface	<b>P/N</b>	Part Number
<b>Hz</b>	Hertz	<b>SN</b>	Serial Number
<b>IFU</b>	Information for Use	<b>SW</b>	Software
<b>IPA</b>	Isopropyl Alcohol	<b>US</b>	United States
<b>IQ</b>	Installation Qualification	<b>USB</b>	Universal Serial Bus
<b>Kg</b>	Kilogram	<b>V</b>	Volts
<b>kHz</b>	Kilohertz	<b>WEEE</b>	Waste Electrical and Electronic Equipment

## GLOSSARY OF SYMBOLS

The following international symbols may be displayed on the shipping container, on the Cellometer Auto T4 device label or in this user manual.

	Keep Dry ISO0626 per EN ISO 15223-1. Located on shipping container.		This End Up ISO0623 per EN ISO 15223-1. Located on shipping container.		Fragile, Handle with Care ISO0621 per EN ISO 15223-1. Located on shipping container.
	FCC Part 15 Supplier Declaration of Conformity. Located in user manual.		Declaration of Conformity to Medical Device Directive 93/42/EEC. Located on SN label and in user manual.		ISO 9001:2015 Certified
	Follow Instructions for Use. Located in user manual.		On—Power Connection to Mains. Located on instrument's Power Switch.		Off—Power Disconnection from Mains. Located on instrument's Power Switch.
	Polarity DC Power Connector IEC 60878. Located on SN label.		Serial Number ISO2498 per EN ISO 15223-1. Located on SN label.		Manufacturer ISO3082 per EN ISO 15223-1. Located on SN label.
	WEEE per EN50419. Located on SN label.				

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# Chapter 2. Equipment Safety

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As with any equipment that involves moving parts, there are potential hazards involved in the operation and maintenance of this instrument. This chapter identifies potential hazards and suggests precautions to avoid them.

## POTENTIAL HAZARDS

This section describes instrument safety features designed to minimize potential hazards. Before using the system, familiarize yourself with this information.



**WARNING: No modification of this equipment is allowed. Modification of equipment can result in improper operation causing possible injury.**

In the United States, the facility operating the instrument should follow all OSHA Manual lines and applicable ANSI standards for the safe use of this instrument.

Customer and operator agree that it is their sole responsibility to fully understand and comply with local, state, and federal laws, rules and regulations in the use of this system.

Cables and accessories not specified within the instructions for use with this instrument are not authorized. Using other cables and/or accessories may adversely impact safety and performance.

**Note:** The Auto T4 instrument should not be used adjacent to or stacked with other equipment unless specified by Nexcelom. If the system must be used adjacent to or stacked with other equipment, then observe the instrument in its configuration to verify instrument operation is normal and functions as expected.

### Electrical Hazard

No part of the exterior housing should be removed. Do *not* open the instrument cover. Contact Nexcelom Support at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)



**WARNING: To avoid the risk of electrical shock, this equipment must only be connected to a grounded electrical outlet.**

**In addition, ensure electrical supply plug is not obstructed and can be reached by users to disconnect the device if necessary.**

## Instrument System Hazard

Read the instructions, warnings and cautions provided with the instrument before using.



**WARNING:** Inspect instruments and cables for breaks, cracks, nicks and other damage before every use. This may be done visually under magnification or with a high voltage insulation testing device. If damaged, do *not* use. Damaged instruments or cables may result in injury to the user.



**CAUTION:** The instrument is designed to accept only one slide at a time. Do *not* attempt to load more than one slide into the sample slot. Doing so will cause an error and could damage the instrument.



**CAUTION:** Do *not* stack equipment on top of the Instrument or place the Instrument on top of electrical equipment. This is an unstable configuration and does not allow for adequate cooling.

In addition, it is recommended that as much distance as possible be provided between the instrument and any equipment emitting a high vibration signature. High levels of vibration may affect clarity of the image being viewed.



**WARNING:** Do *not* remove the instrument cover. Contact Nexcelom Support for assistance at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

## Servicing Hazard

No one other than Nexcelom-authorized personnel may service inside the protective cover of the Auto T4 instrument system.



**CAUTION:** Do not pull the system by the connectors. Toppling of the system or causing damage to the system may result in the instrument no longer functioning as expected.

## SAFETY INFORMATION

Personnel operating and maintaining the Auto T4 instrument should be familiar with the safety information included in this section.

### Safety Protocols

Nexcelom assumes no liability whatsoever for any damage, loss or injury resulting from an application of a product that is not in strict accordance with the instructions provided with the product. Nexcelom also assumes no liability for any damage or injury arising as a result of operator error or mistake, including, but not limited to, injury arising from operator's lack of qualification or as a result of errors or mistakes committed by such operator.

Read all installation and operation instructions contained in this user manual thoroughly before connecting the Auto T4 instrument to the main power connection *prior* to use. The Auto T4 must be set, regulated and used in accordance with the instructions outlined in this user manual. Failure to observe safety warnings and precautions may present a risk.

Only individuals with appropriate safety training and knowledge should operate, assist in the operation of, or perform cleaning and routine maintenance of this instrument. Only the operator should be responsible for system controls during a procedure.

### Safety Features

The Auto T4 Instrument offers several safety features to prevent its misuse or unintentional activation. All personnel who operate the system should be familiar with these safety features.

Contact Nexcelom Support for assistance at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)



### Federal Communications Commission

This device complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This device may not cause harmful interference and (2) this device must accept any interference received, including interference that may cause undesired operation.

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# Chapter 3. Instrument Description

This chapter describes the Auto T4 instrument components, device label and system specifications.

## AUTO T4 COMPONENTS



**Auto Sample Slot:** Chambered slides are inserted through this slot for viewing (accepts only one slide at a time).

**Focus Knob:** Radial dial used to focus the camera.

**Power Switch:** Controls power going to the instrument.

**USB 2.0 Port:** Port used to connect the Auto T4 to the Operating Computer.

**Power Cord Connector Input:** Port used to connect the power supply to the Auto T4.

**Device Label:** Lists the instrument serial number (SN), model, manufacturer and input power requirement.

## DEVICE LABEL



Serial Number



Manufacturer



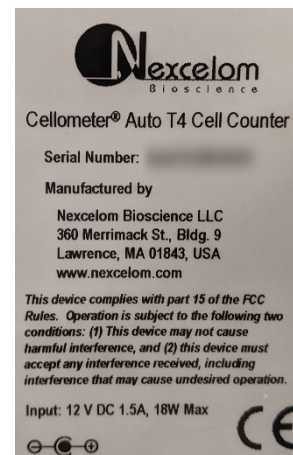
Polarity DC Power Connector



Waste Electrical and Electronic Equipment Directive



Conformité Européenne 93/42/EEC



## SYSTEM SPECIFICATIONS

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 storing count results.

	Cellometer Auto T4 Cell Counter
Channels	Brightfield
Number of Channels	1
Commonly Used Compatible Dyes	Trypan Blue
Counting Speed Per Sample	<30sec
Volume (per chamber)	20 $\mu$ L
Cell Size/Diameter Range	5 - 60 $\mu$ m (most mammalian cells)
Concentration Range	$1 \times 10^5$ – $1 \times 10^7$
Software	Cellometer Auto Pattern-recognition software to quickly and accurately decluster, identify and count individual cells
Weight	10.4 lbs (4.7 kg)
Width	3.5" (8.9 cm)
Depth	4.2" (10.7 cm)
Height	12.6" (32.0 cm)
Input to Power Adapter	100-240 VAC, 50/60 Hz, 0.5A
Output to Instrument	12 VDC, 1.5A

# Chapter 4. Unpacking and Site Preparation

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This chapter presents site preparation facility requirements for setting up the Auto T4 system and transporting instructions if the instrument must be moved to another location.

## UNPACKING THE INSTRUMENT

Congratulations on your purchase of one of the best declustering cell counters available! Please ensure you have secured the space required to set up the Auto T4. Visit the [Cellometer Auto T4](#) page on our website, click the **Resources** tab and scroll down to find *Training Videos* for unboxing and getting started with the instrument.

Our staff would be happy to help you set up your Auto T4. Contact Nexcelom Support for assistance at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

1. Inspect the package to ensure no damage has occurred during shipping, if applicable.  
**Contact Nexcelom Support if damage has visibly affected the instrument.**
2. Ensure the box is facing up (i.e., *This End Up* symbol is facing in the right direction). If not, carefully turn the box right side up. *Box will weigh approximately 10 lbs (4.7 Kgs).*
3. Open the outer box.
4. Remove protective packaging.
5. Remove the inner box.
6. Open the secondary box to remove the Power Cord, USB Computer Cable and Software USB.
7. Remove the Auto T4.
8. Place the Auto T4 in the prepared area (see [Facility Requirements](#) on page 14).
9. Remove the protective seals around the Auto T4.
10. Insert the Power Cord Connector into the Auto T4.
11. Plug the Power Cord into a surge protector (recommended) or power outlet.
12. Obtain the USB 2.0 Computer Cable.
13. If you have purchased a Nexcelom Laptop or have installed the Cellometer software on a laptop meeting Nexcelom minimum specifications, connect the Auto T4 to the laptop using the USB 2.0 Computer Cable.  
**Note:** If you have *not yet* installed the software, do *not* connect the instrument to the computer until directed during software installation.
14. Turn the Auto T4 Power Switch to the ON position. The system is now ready for use.



### Auto T4 IQ/OQ Validation

Nexcelom Bioscience has designed an *Installation Qualification/Operation Qualification (IQ/OQ)* validation specifically for the Cellometer Auto T4. Our experienced Support team is available to assist your organization during set up of the instrument and while performing the IQ/OQ validation. Contact Nexcelom Support for more information at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

## SITE PREPARATION

### Facility Requirements

Instrument must be plugged directly into a surge protector (recommended) or power outlet. Ensure all cables are free from kinks or tangles *prior* to starting the Auto T4.

Due to how the instrument is used with liquids, the instrument should be placed on a level surface.

Keep the area around the Auto T4 clean between, during and post operation.

Electrical requirements for the Auto T4 are as follows:

- 0.5 AMP service
- Wall receptacle voltage between 100 to 240 VAC
- 50/60 Hz

**Note:** Voltage requirements vary per region. For international customers, ensure that Power Cord meets local regulations or use a suitable replacement.

**Note:** The instrument can be disconnected from the mains by disconnecting the Power Cord from the mains plug or appliance coupler.



**CAUTION:** Do *not* position the device such so that it is difficult to disconnect from power main.

### Transporting the Instrument

Prior to transporting the instrument by vehicle, disconnect the main from the wall and package all accessories and consumables individually and safely, ensuring cables are also protectively wrapped. Refer to instrument dimensions to ensure vehicle to be used for transporting meets these requirements.

When the instrument is loaded into the vehicle, it should be secured in such a way to prevent the instrument or components from shifting during transport.



**WARNING:** Care should be taken while moving or transporting the instrument to prevent damage to the instrument and/or possible injury.

### Disposal of Waste Electrical and Electronic Equipment



To comply with European Commission Directive 2012/19/EU on Waste Electrical and Electronic Equipment (WEEE) and other country and state regulations, do *not* dispose of this equipment in any location other than designated waste locations. Contact Nexcelom Support for proper product disposal at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

# Chapter 5. Operation

This chapter describes installing the software and steps to be performed when using instrument for the first time.



**WARNING:** Never force the door open or place a foreign object inside the Auto Sample Slot. Should anything get stuck or spill inside the sample slot, power OFF the instrument and contact Nexcelom Support for service at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

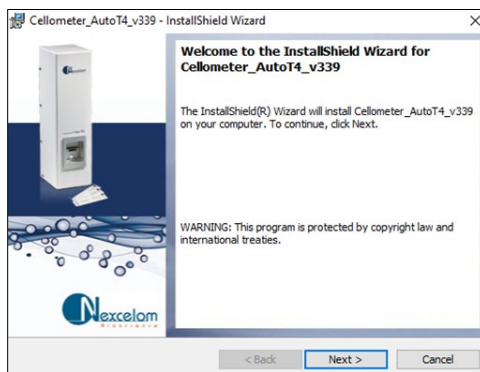
## INSTALLING THE SOFTWARE

*If the Operating Computer was purchased from Nexcelom along with the instrument, the software was pre-installed on the laptop and you can skip this section. Continue to [Using Instrument for the First Time](#) on page 17.*

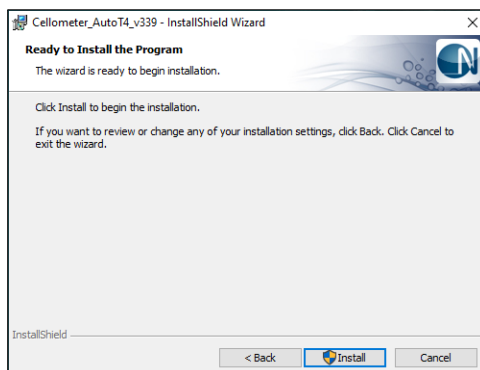
**Note:** Ensure that instrument is *not* connected to the computer during software installation until specified.

To install the Cellometer software on a computer that was *not* purchased from Nexcelom, follow these steps.

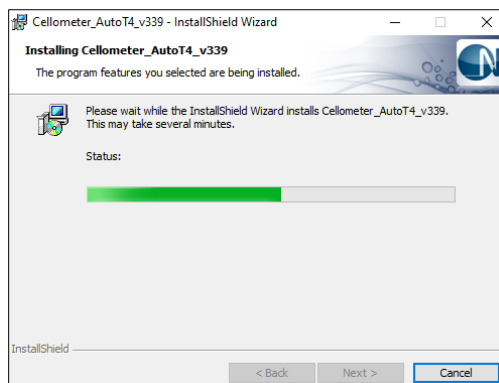
1. Insert Software USB Drive that was included with the instrument into USB port of the Operating Computer.
2. Extract files contained on the USB to the computer desktop.
3. On the desktop of Operating Computer, double-click the **Cellometer\_version.exe** file (where **version** represents the version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



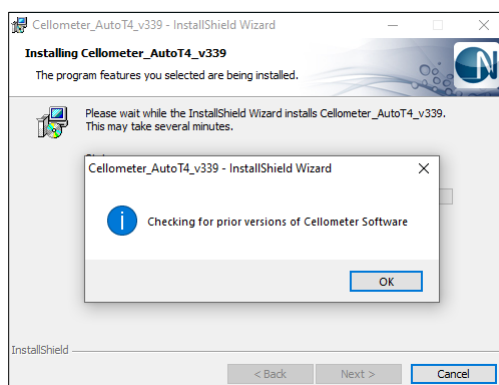
4. Click **Next** to begin the Auto T4 software installation.



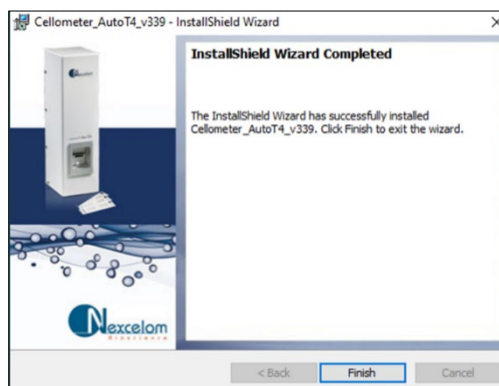
- Click **Install** to install the instrument software on the hard drive of the Operating Computer. A screen containing prerequisite software to be installed on your computer may be displayed. Click **Install** to continue.



- When prompted, click **OK** to check for prior versions of Cellometer Auto T4 software.



- Click **Finish** to exit the installer.



- Confirm that **Cellometer Auto** software icon now appears on desktop of Operating Computer.
- Remove the Software USB and store it according to personal/company guidelines.
- Plug power cord for the Auto T4 instrument into a surge protector (recommended) or outlet and power ON.
- 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 Software

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 back of the instrument.



 The dialog box has a title bar that says 'New Instrument Detected' with a close button (X) on the right. The main text says 'New Instrument detected.' followed by 'Please enter the "Serial Number" located on the back of the instrument.' Below this, there are three input fields with labels 'AutoXXX', 'x0x', and 'xxxx' above them. The first field contains 'Autoxx', the second contains 'xxx', and the third contains 'xxxx'. To the right of these fields, there is an 'Example:' section showing two examples: '"AutoT4-006-0101"' and '"AutoM10-003-0101"'. Below the input fields, there is a label 'Nickname (optional)' above a text input field. To the right of this field, there is another 'Example:' section showing '"Time Saver"'. At the bottom center, there is a 'Done' button.

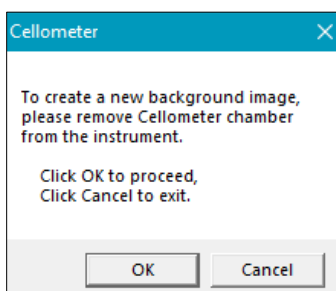
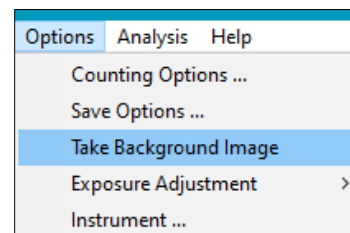
3. Click **Done** to save the instrument Serial Number.
4. A pop-up message will appear indicating a background image needs to be taken before counting. Click **OK** to continue.

 The dialog box has a title bar that says 'Cellometer' with a close button (X) on the right. The main text says 'No background image found.' followed by 'You must take a background image before counting.' Below this, it says 'From the Options menu select: "Take background image"'. At the bottom right, there is an 'OK' button.

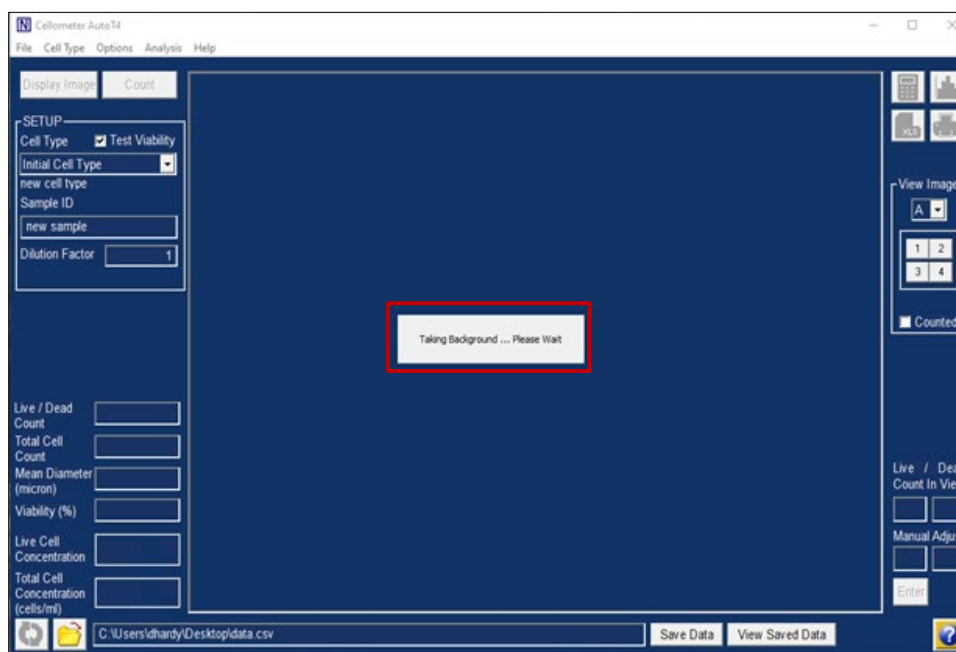
## Taking a Background Image

After initial setup of the instrument, you must take a background image of the system by itself (without the cell counting chambered 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 the *Take Background Image* option.
3. Remove the chambered slide from the instrument, if applicable.

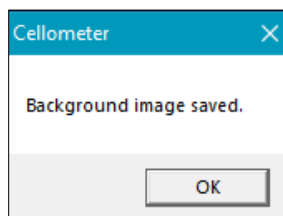


4. Click **OK** to confirm the Auto Sample Slot is empty.
5. The system automatically takes the background image.

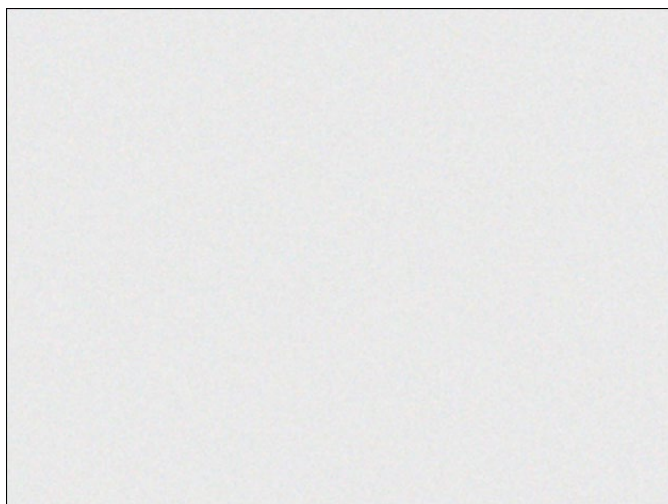




6. Once the image is complete, a pop-up message indicates the background image has been saved. Click **OK**.



7. Click the **Display Image** button to view the background image taken.
8. Confirm that the background is a uniform gray color.



If there is any discoloration (e.g., light/dark areas) of the image, contact Nexcelom Support for assistance at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

9. Return to the Home screen to begin counting samples.

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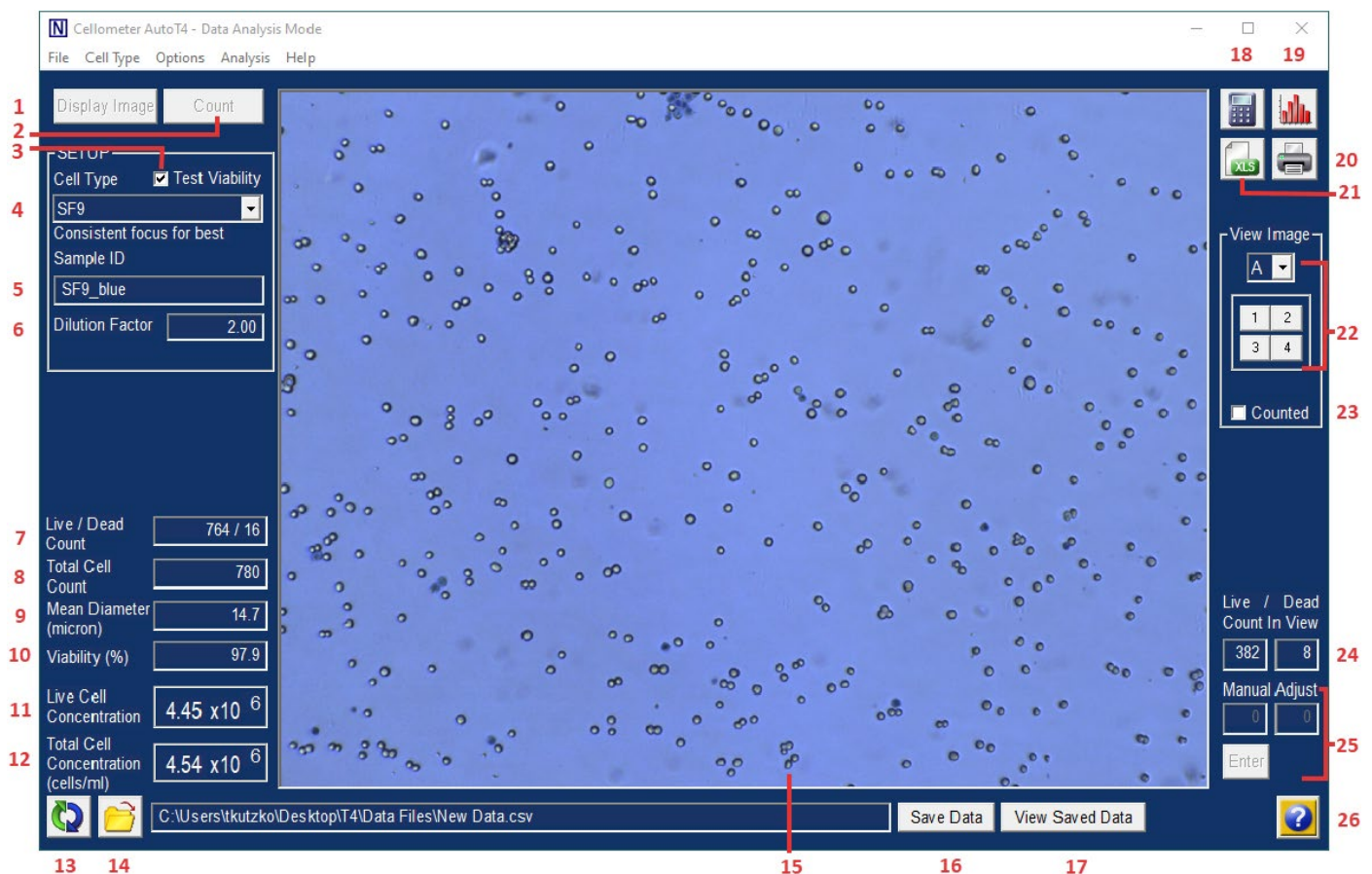
# Chapter 6. Software GUI Elements

This chapter presents key graphical user interface (GUI) menus and screens available in the Auto T4 software.








**Note:** The Auto T4 software used to run the instrument must be installed on the Operating Computer before you can use the GUI. See [Installing the Software](#) on page 15 for details. *If the Operating Computer was purchased from Nexcelom along with the instrument, the software was pre-installed on the laptop and is ready for use.*

## HOME SCREEN

The *Home Screen* (also referred to as the main screen) in the Auto T4 software is described below.



- 1 **Display Image Button** – Shows cells currently in the counting chamber. *Not enabled if a saved image has been loaded for display.*
- 2 **Count Button** – Initiates counting procedure. *Not enabled if a saved image has been loaded for display.*
- 3 **Test Viability** – Indicates that a viability stain has been used.
- 4 **Cell Type** – Identifies cell type parameters to be used when counting.

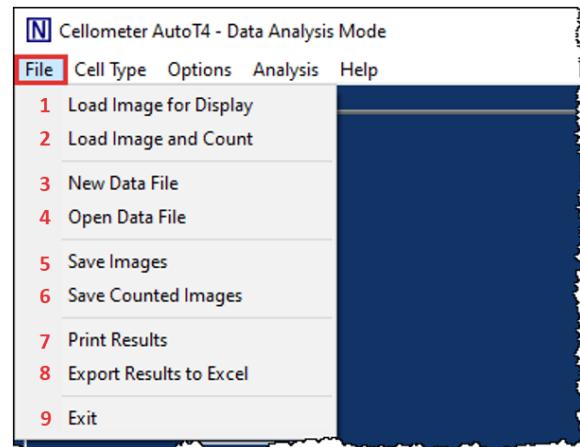
- 5 **Sample ID** – Allows entry of a unique sample identifier.
- 6 **Dilution Factor** – Allows entry of final dilution factor for cell sample to calculate accurate concentration.
- 7 **Live/Dead Count** – Displays numbers of live and dead cells found. *Displayed only if **Test Viability** is selected.*
- 8 **Cell Count** or **Total Cell Count** – Displays total number of cells counted in the images taken. *Display of field toggles based on whether **Test Viability** is selected.*
- 9 **Mean Diameter (micron)** – Identifies mean diameter of counted cells in microns.
- 10 **Viability (%)** – Identifies percent viability of cell sample. *Displayed only if **Test Viability** is selected.*
- 11 **Live Cell Concentration** – Indicates live cell concentration (in cells/mL) calculated using the live cell count and dilution factor. *Displayed only if **Test Viability** is selected.*
- 12 **Cell Concentration** or **Total Cell Concentration (Cells/mL)** – Indicates cell concentration/total cell concentration calculated using the cell count/total cell count and dilution factor. *Display of field toggles based on whether **Test Viability** is selected.*
- 13 **Recount Icon**  – Recounts cell sample using the previously taken images.
- 14 **Select Data File Icon**  – Opens Windows File Explorer so users can indicate where count result data and images should be saved.
- 15 **Image Area** – Displays image 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 used to calculate volume needed to adjust concentration; can only be used after a count is performed.
- 19 **Cell Size Analysis Icon**  – Displays a histogram of cell diameter based on cells counted in the images using the *Cell Size Analysis* tool; can only be used after a count has been performed.
- 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 via drop-down; 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. *Display of field toggles based on whether **Test Viability** is selected.*
- 25 **Manual Adjust** or **Live/Dead Manual Adjust** – Enables users to manually adjust cell numbers by entering a count of cells in view. *Display of field toggles based on whether **Test Viability** is selected.*
- 26 **Help Icon**  – Displays window providing users with access to online help contents and resources, instructions for submitting a support ticket, and Nexcelom contact information.

## AVAILABLE MENUS

The following menus are available in the *Menu Bar* displayed across the top of the Home screen.

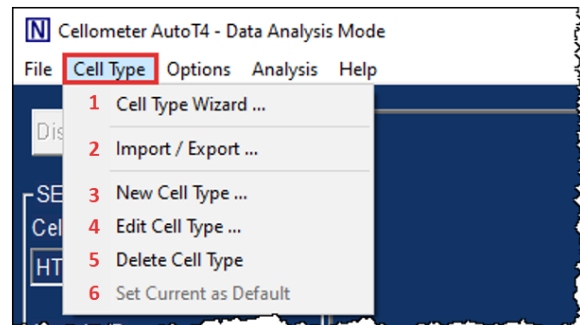
### File Menu

- 1 Load a previously saved image and display it.
- 2 Load a previously saved image and count it.
- 3 Choose a new data file.
- 4 Open a previously saved data file.
- 5 Save images the instrument has taken.
- 6 Save counted images with the graphic overlay.
- 7 Print results of the last count.
- 8 Export results of the last count to MS Excel.
- 9 Exit the software.



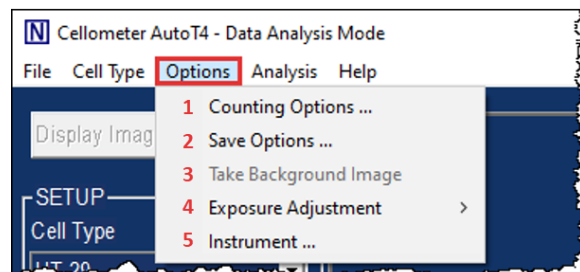
### Cell Type Menu

- 1 Launch the Cell Type Wizard.
- 2 Import/Export cell types.
- 3 Create a new cell type.
- 4 Edit the currently selected cell type.
- 5 Delete the currently selected cell type.
- 6 Set the current settings as default.





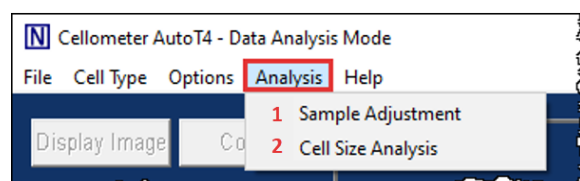
### Options Menu

- 1 Edit the counting options.
- 2 Edit the save options.
- 3 Take new background image.
- 4 Adjust brightfield exposure.
- 5 View instrument details.

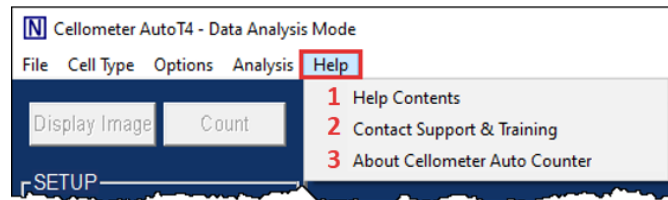


### Analysis Menu


- 1 Open Sample Adjustment tool; *also accessible via the  icon from the Home screen.*
- 2 Open Cell Size Analysis tool; *also accessible via the  icon from the Home screen.*

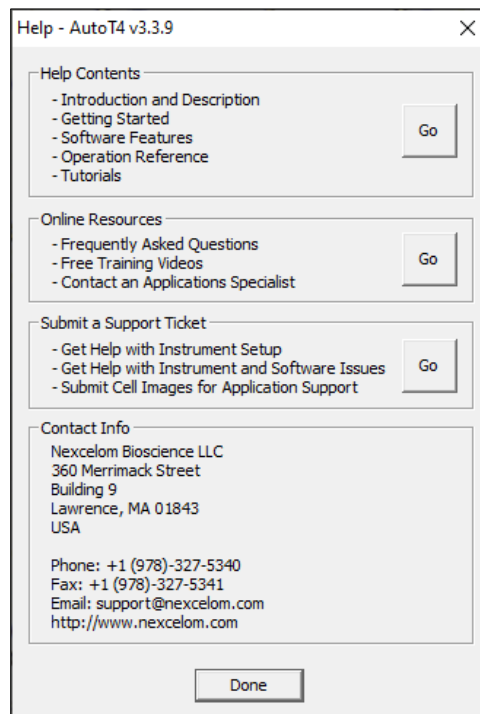


## Help Menu



- 1 The online help consists of instructions for operating Auto T4.
- 2 Launches Nexcelom's online resources page containing video tutorials, instructions on how to submit a support ticket including sample images, and access to Frequently Asked Questions/Application Notes (an internet connection is required when using this option).
- 3 Displays version number of the Cellometer Auto T4 software currently installed.

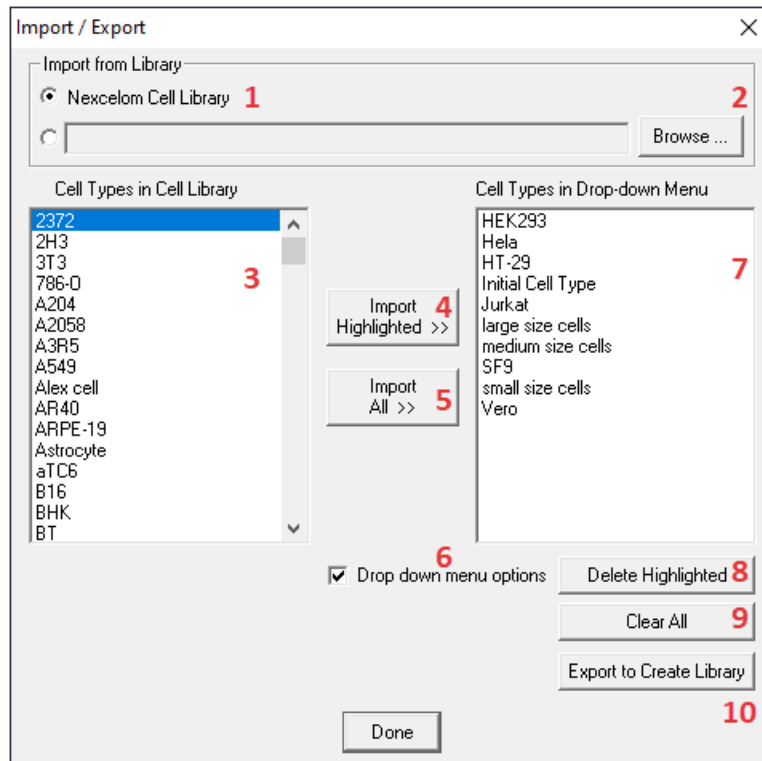
**Note:** Alternatively, clicking the **Help** icon  located in the bottom right corner of the Home screen also displays online help contents and resources, a link for submitting a Support ticket, and Nexcelom contact information.



## IMPORT/EXPORT CELL TYPE SCREEN

*Cell Types* define image analysis parameters used in cell sample detection. The *Import/Export Cell Type Screen* is used to import either standard cell types from the *Nexcelom Cell Library* installed with the Auto T4 software or custom cell types from an external network location.

Once a library is selected, you can highlight cell types from the library (displayed in the pane on the left) and import them to appear in the **Cell Types** drop-down menu available on the Home screen (displayed in the pane on the right). Cell types selected to appear in the drop-down menu can also be exported to create a new library.



- 1 Selects **Nexcelom Cell Library** to display Nexcelom cell types in the *Cell Types in Cell Library* pane.
- 2 Selects a custom library to display available cell types in the *Cell Types in Cell Library* pane. If no library name is displayed, click the **Browse** button and navigate to an external cell type library.
- 3 List of cell types in the currently selected Cell Library.
- 4 Imports the highlighted cell type (from the left pane) into the drop-down menu (right pane).
- 5 Imports all cell types (from the left pane) into the drop-down menu (right pane).
- 6 Select to display drop-down menu options.
- 7 List of cell types to be displayed in the drop-down menu.
- 8 Deletes the highlighted cell type in the drop-down menu (displayed in the right pane).
- 9 Clears all cell types in the drop-down menu (displayed in the right pane).
- 10 Exports drop-down menu options (displayed in the right pane) to create a new Cell Library.

## Cell Type Drop-down Defaults

The Auto T4 displays several commonly known cell types that have been pre-selected to appear in the **Cell Type** drop-down menu available on the Home screen as initial defaults. These cell types are listed below:

- **HEK293** – Embryonic kidney cell line
- **Hela** – Immortal cell line derived from cervical cancer cells
- **HT-29** – Human colorectal adenocarcinoma cell line
- **Initial Cell Type** – Generic cell type with default settings
- **Jurkat** – Immortal cell line of Human T lymphocytes
- **Large Size Cells** – Generic cell type with a large cell diameter range
- **Medium Size Cells** – Generic cell type with a medium cell diameter range
- **SF9** – Clonal isolate of *Spodoptera frugiperda* cells
- **Small Size Cells** – Generic cell type with a small cell diameter range
- **Vero** – Isolate of kidney epithelial cells

Users can import additional cell types to appear in this drop-down as necessary from the Nexcelom Cell Library. See [Nexcelom Cell Library](#) on page 49 for a complete list of available cell types.

## Importing Cell Types

Used to import cell types from the Nexcelom Cell Library or from external custom libraries. Individual cell types can then be selected to appear in the **Cell Types** drop-down menu displayed on the Home screen.

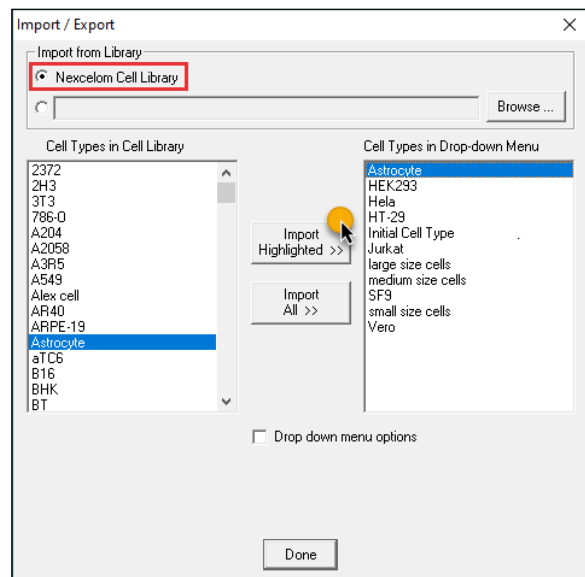
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### FROM NEXCELOM CELL LIBRARY

The **Nexcelom Cell Library** is available by default and if selected in the Import/Export Cell Type screen, available cell types are displayed in the *Cell Types in Cell Library* pane.

1. In the *Import from Library* section of the Import/Export Cell Type screen, ensure that the **Nexcelom Cell Library** radio button is selected.
2. In the *Cell Types in Cell Library* pane (displayed on the left), highlight a cell type to be imported.
3. Click **Import Highlighted >>** to import highlighted cell type to appear in the *Cell Types in Drop-down Menu* pane (displayed on the right).

**Note:** Although **Import All >>** can be used to import *all* cell types, it is *not* recommended if the size of the cell library will make the **Cell Types** drop-down menu in the Home screen difficult to use.

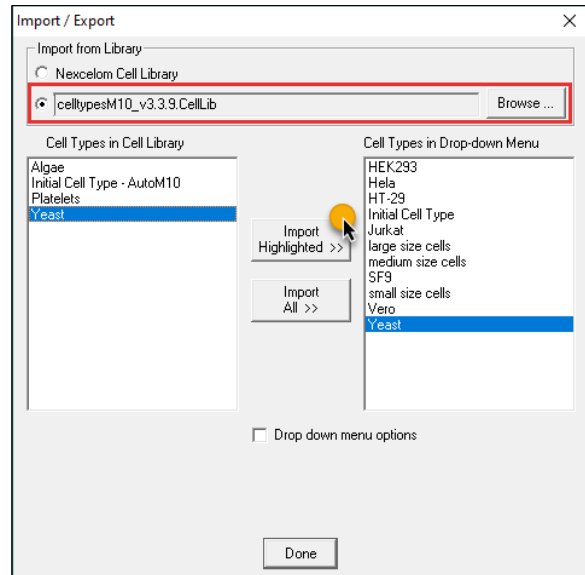




## FROM CUSTOM LIBRARIES

1. In the *Import from Library* section of the Import/Export Cell Type screen, click the custom cell library radio button to choose a custom library (if available) or click **Browse** to navigate to folder containing a custom library and double-click on a library name to select it. *This text box will appear as blank if a custom cell library has not yet been selected.*
2. Ensure that cell types from the selected library appear in the *Cell Types in Cell Library* pane.
3. In the *Cell Types in Cell Library* pane (displayed on the left), highlight a cell type to be imported.
4. Click **Import Highlighted >>** to import highlighted cell type to appear in the *Cell Types in Drop-down Menu* pane (displayed on the right).

**Note:** If the custom library selected is of a manageable size for the Cell Types drop-down menu, **Import All >>** can be used to import *all* cell types from the library.

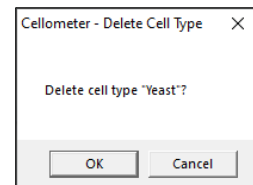
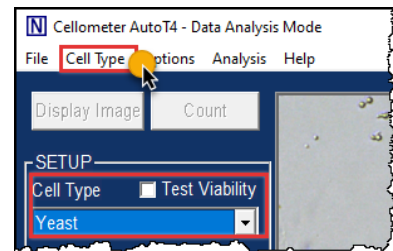


## Deleting Cell Types

## FROM HOME SCREEN

1. Use the *Cell Type* drop-down menu to select the cell type to be deleted.
2. Open the Cell Type Menu in the menu bar and select the *Delete Cell Type* option.
3. Click **Delete Cell Type**.
4. Click **OK** to confirm the deletion.
5. Confirm cell type has been removed from *Cell Type* drop-down menu.

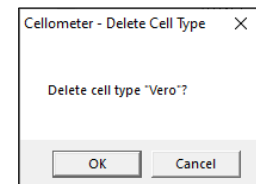
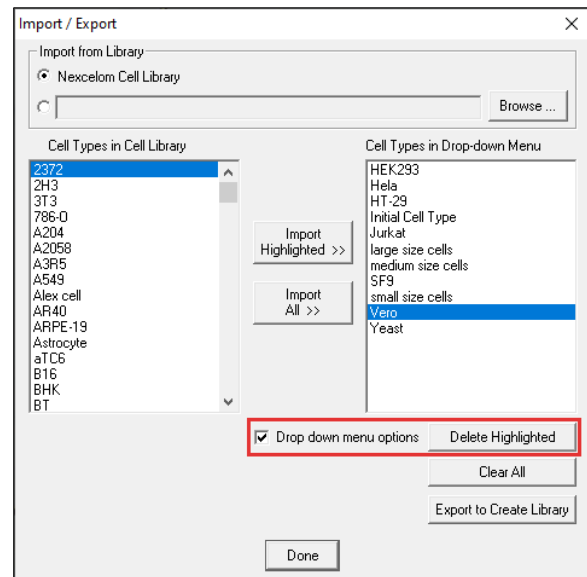
**Note:** If cell type deleted was from the Nexcelom Cell Library, it can be re-imported if necessary. If cell type was from a custom library or created using the Auto T4, it may be permanently deleted unless cell type was exported and saved prior to deletion.



## FROM IMPORT/EXPORT CELL TYPE SCREEN

1. From the Home screen, open the Cell Type Menu in the menu bar and select the *Import/Export...* option.
2. In the *Cell Types in Drop-Down Menu pane* (displayed on the right), highlight the cell type to be deleted.
3. Click the **Drop-down Menu Options** checkbox to display additional options.
4. Click the **Delete Highlighted** button.
5. Click **OK** to confirm the deletion.
6. Confirm cell type has been removed from *Cell Type* drop-down menu.

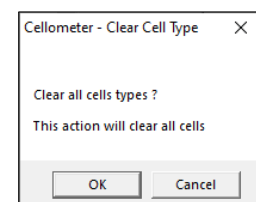
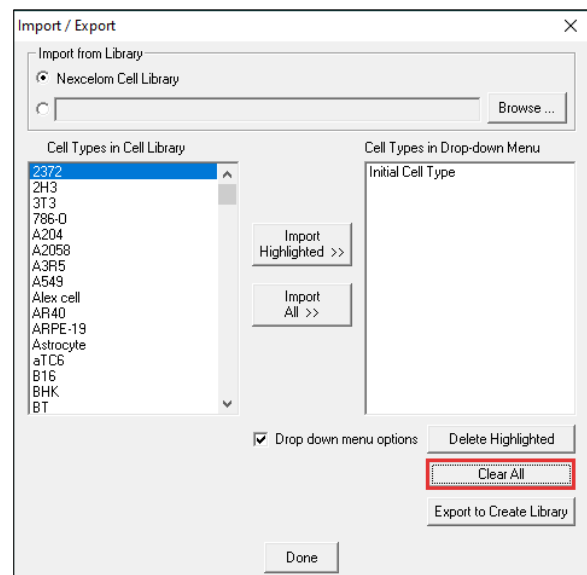
**Note:** If cell type deleted was from the Nexcelom Cell Library, it can be re-imported if necessary. If cell type was from a custom library or created using the Auto T4, it may be permanently deleted unless cell type was exported and saved prior to deletion.



## Clearing Cell Types from Drop-down

1. From the Home screen, open the Cell Type Menu in the menu bar and select the *Import/Export...* option.
2. To clear all cell types from the *Cell Types in Drop-down Menu pane* (displayed on the right), select the **Drop-down Menu Options** check box.
3. Click the **Clear All** button.
4. Click **OK** to confirm the clearing of all cell types. *Only the default Initial Cell Type will remain.*

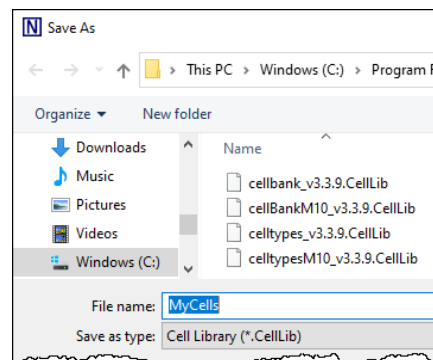
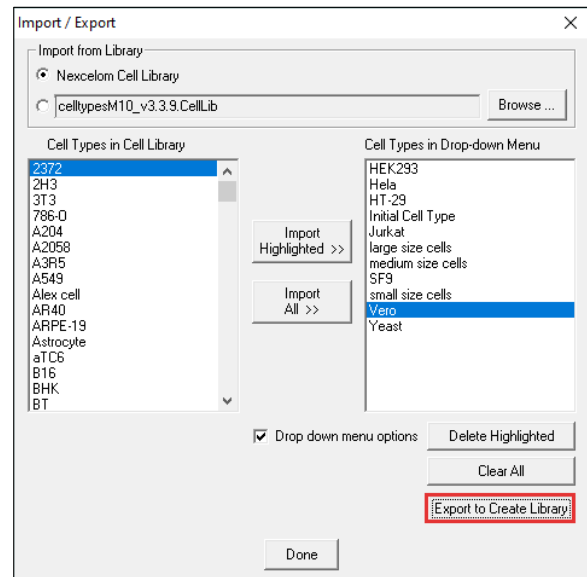
**Note:** If cell types cleared were from the Nexcelom Cell Library, they can be re-imported if necessary. If cell types were from a custom library or created using the Auto T4, they may be permanently deleted unless cell types were exported and saved prior to deletion.



## Exporting Cell Types to a Library

Users can export cell types selected to appear in the **Cell Type** drop-down menu available on the Main Screen (displayed in the right pane) to create a custom Cell Library.

1. From the Home screen, open the Cell Type Menu in the menu bar and select the *Import/Export...* option.
2. To export cell types listed in the *Cell Types in Drop-down Menu* pane (displayed on the right), select the **Drop-down Menu Options** check box.
3. Click the **Export to Create Library** button.
4. Click **OK** to confirm save.
5. Save file in designated folder.
6. Click the **Done** button to return to the Setup screen.



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# Chapter 7. Sample Preparation

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## PREPARING CELLOMETER CHAMBERED SLIDES

1. For SD100 slides, remove the protective film from both sides of the slide. *PD100 slides do not have this film.*

**Note:** It may be difficult to peel film from the slide. Some clients use tape to grab the film and then pull it off. Do *not* touch or write on the clear optical window areas of the slide.

Top of Slide



Bottom of Slide



2. Place the Cellometer disposable chambered slide on a fresh Kimwipe.
3. To prepare two samples at once, label the individual chambers (e.g., *Chamber 1* and *Chamber 2*) in the white margin of each chamber. Take care to ensure the clear portion of the counting chamber is not touched.

**Note:** Both chambers of the slide do *not* need to be loaded with the same sample. Two different samples can be used on a single slide.

## LOADING SAMPLE INTO COUNTING CHAMBER

Cellometer chambered slides consist of two independent counting chambers manufactured to a precisely-controlled volume. A cell suspension of 20  $\mu$ L is pipetted into a chamber through one of the induction ports.

1. To prepare the sample for counting, invert the tube containing your sample 10 times, then pipette up and down 10 times. This will help evenly suspend cells. Do *not* shake sample as it may damage cell membranes.

**Note:** If testing for cell viability (*optional*), stain the sample using a 1:1 ratio with 0.2% trypan blue (or other chosen viability stain) and mix the stain by pipetting up and down 10 times *before* performing Step 1, above.

*When preparing a sample to test for cell viability, ensure that the trypan blue stock concentration used is 0.2%. If stock concentration is 0.4%, it is recommended you dilute it with a balanced salt buffer (e.g., PBS) and filter diluted solution with a 0.2 micron filter before adding stain to your sample.*

2. Immediately transfer 20  $\mu$ L of sample into a counting chamber by pipetting directly onto sample induction port. *There are two counting chambers per slide; each chamber may be loaded with a different sample.*
3. Hold the loaded chamber in the white area and take care to stay away from the clear optical window.



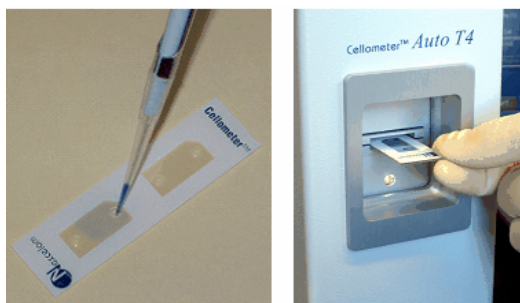
Load the counting chamber by touching the pipette tip to a sample introduction port and then slowly pipette the entire 20  $\mu$ L sample at once. Capillary force automatically spreads the cell sample within the counting chamber. The chambers can hold separate samples as there is no mixing between the two chambers on the slide.

## Chapter 8. Counting and Analysis Workflow

This chapter presents basic workflow for counting cells/beads using the Auto T4, including best practices and tips. Sample images are provided as a reference for many common cell types.

### PERFORM CELL COUNTING

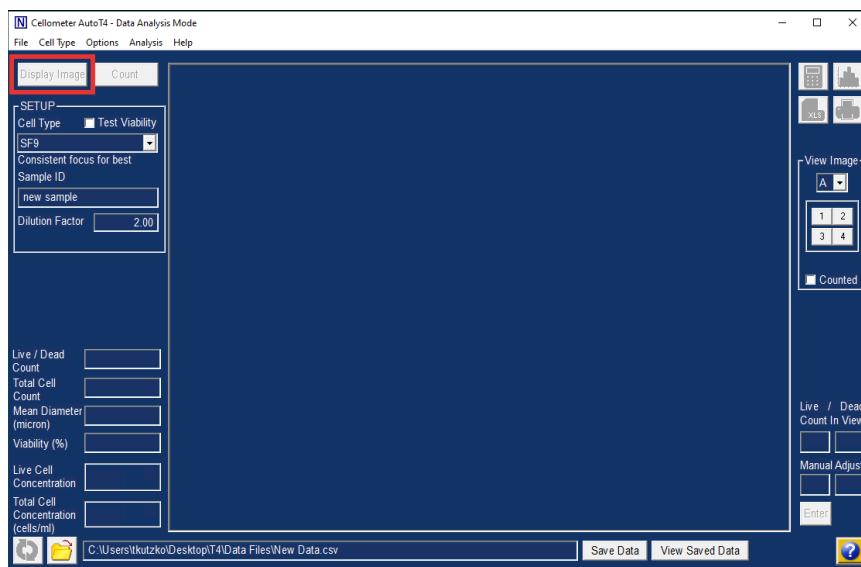
1. Power ON the instrument and from the desktop of the Operating Computer, launch the Auto T4 software.
2. Slowly Pipette 20  $\mu$ l of the cell sample into counting chamber. See [Preparing Cellometer Chambered Slides](#) on page 31 for details.



3. Load the chambered slide into the Auto T4 by inserting the counting chamber that contains the cell sample into Auto Sample Slot until touching the internal stop. *The chamber inside the instrument will be counted.*

**Note:** If testing for cell viability, you must stain the sample before loading it into the slide chamber.

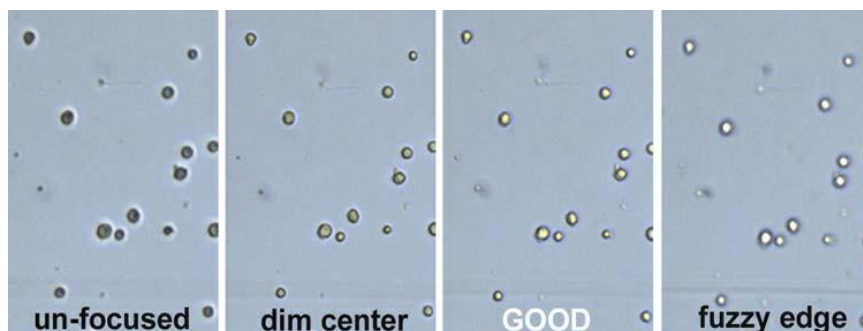
4. Click the **Display Image** button.



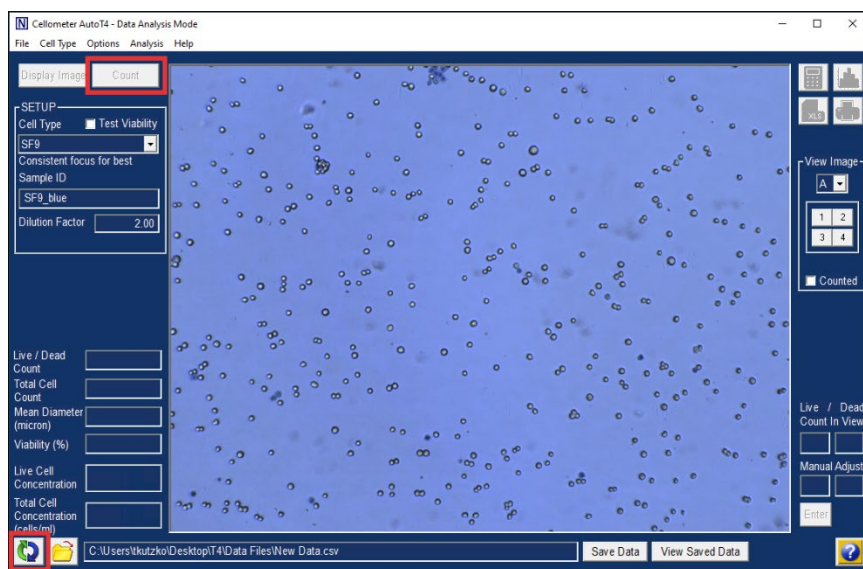
**Note:** When software is in *Data Analysis Mode*, the **Display Image** button will *not* be enabled. From the File menu, select *Load Image for Display* or *Load Image and Count*, then navigate to a previously saved image.




- Slowly turn the Focus Knob on side of the instrument until good focus is achieved to ensure accurate counts. Live cells will have a bright center and a dark, crisp clearly defined edge as shown in the examples below.



- In the *Setup* area of the screen, choose the **Cell Type** from the drop-down list, enter a **Sample ID** and ensure the **Dilution Factor** is accurate for the sample.
- Click the **Count** button while the cell image is displayed.

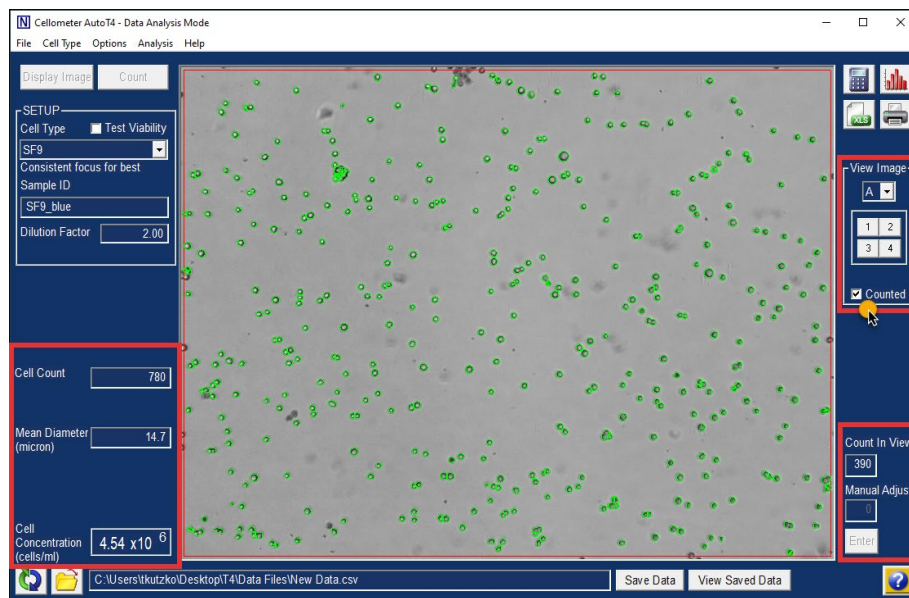


**Note:** When software is in *Data Analysis Mode*, the **Count** button will *not* be enabled. Click the **Recount** icon  to re-analyze the captured image.



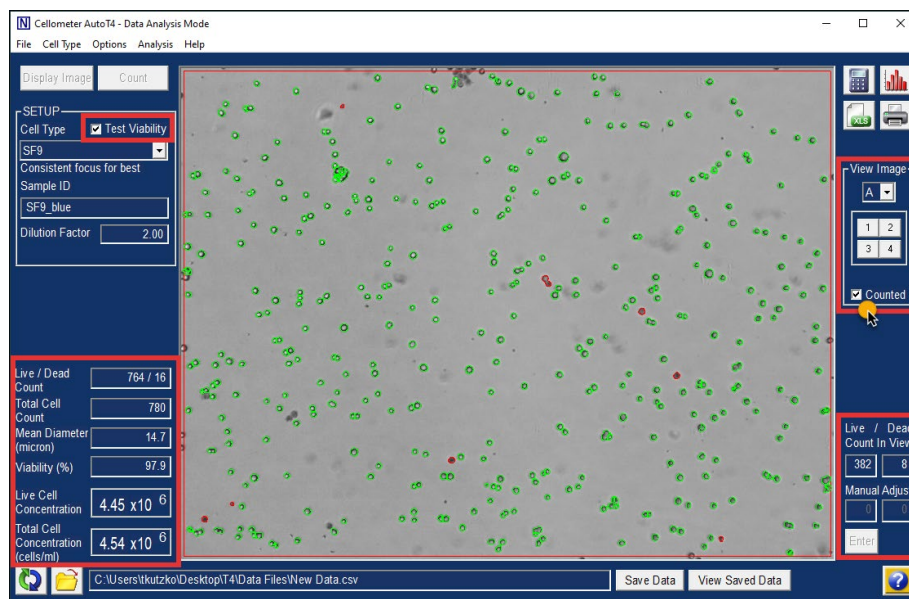
## REVIEW COUNT RESULTS

Once counting is complete, count 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 the counting chamber. 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 were larger than the specified cell diameter).



**Note:** When viewing counted cells, a red line will appear along edges of the Viewing Pane identifying the outer perimeter of the counting area. Any cells appearing on or outside of this red line are *not* counted.


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

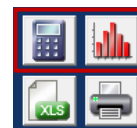


## Best Practices and Workflow Tips

- The progress bar at the bottom of the software panel indicates counting is in progress. A numeric counter also displays updated cell counts.
- Count results displayed in the bottom left corner of the screen represent the entire counting chamber and include *Cell Count*, *Mean Diameter (micron)* and *Cell Concentration (cells/mL)*.
- Count results displayed in the bottom right corner of the screen represent only the cells seen in the current view (i.e., displayed in the image area). Use the *View Image controls* to change the current view.
- When the **Test Viability** check box in the *Setup* area is selected, additional count results are displayed including live and dead cell counts (i.e., *Live/Dead Count*, *Total Cell Count*, *Live Cell Concentration*, *Total Cell Concentration*). Viability percentage is calculated with live cell count as a percentage of total cell count, including live and dead cells. *Viability testing can only be done with the addition of a Brightfield live/dead stain like trypan blue is added to the cell sample.*
- If cell counting optimization is required, you can customize cell type parameter settings using the *Edit Cell Type* option under the Cell Type drop-down menu. See [Editing Cell Types](#) on page 41 for details.

**Note:** If you need help optimizing cell type parameter settings, contact Nexcelom Support for assistance at +1 (978) 327-5340 or email: [support@nexcelom.com](mailto:support@nexcelom.com)


- After modifying cell type parameters, click the **Recount Image** icon  to recount cells in the image.
- Data analysis features include *Sample Adjustment* and *Cell Size Analysis* tools. Based on the measured cell concentration, you can calculate the sample adjustment to achieve a target concentration or cell number. In addition, cell size data for the sample can be displayed using a histogram. See [View Cell Size Analysis](#) on page 37 and [Calculate Sample Adjustment](#) on page 38 for details.



## Saving Data and Images

- To save count result data (as a .txt file) while viewing individual cell samples, click the **Save Data** button located at the bottom of the screen. See [Manually Saving Data](#) on page 51 for details. Once saved, you can click the **View Saved Data** button to view it. Use the *New Data File* option on the File Menu to change the selected data file. See [Automatically Saving Data](#) on page 52 to automatically save data after each count.
- To save count result images (as .png and .jpg files) while viewing individual cell samples, use the *Save Images* or *Save Counted Images* options on the File Menu and navigate to a destination folder for saving image files. See [Manually Saving Images](#) on page 51 for details. *All images captured for the sample during counting (i.e., A, B) will be saved as specified. See [Automatically Saving Images](#) on page 52 to automatically save images after each count.*


## Printing Data

Results can be printed after performing a count by clicking the **Print** icon  and selecting the name for a printer (e.g., may include applications such as *Microsoft Print to PDF*, *Microsoft XPS Document Writer*, *OneNote (Desktop)* and *SnagIt 2021*).

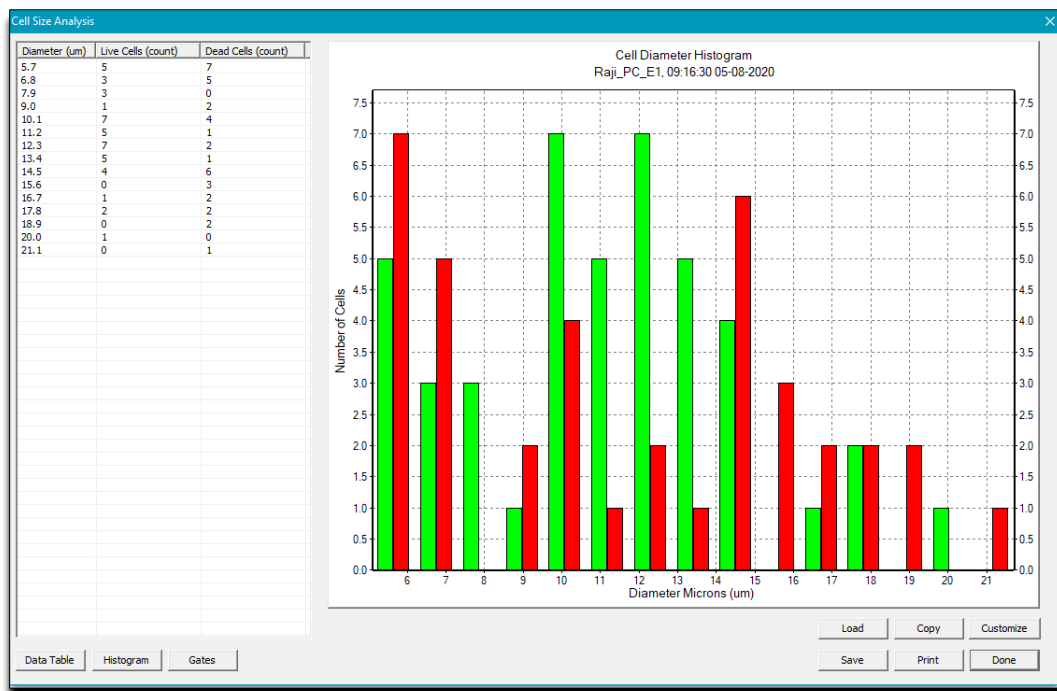
Alternatively, data can be printed by selecting the *Print Results* option from the File Menu.

## VIEW CELL SIZE ANALYSIS

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. By overlaying data from multiple samples into a single histogram, users can create an analysis of the change in cell diameter size over time.

To use the *Cell Size Analysis* tool, you must first perform a count on either a live or saved image to display the diameter for each object found, as well as the mean diameter for cells in your sample (including dead cells if **Test Viability** was selected). Click on the **Cell Size Analysis** icon  to plot this information into a *Cell Diameter Histogram* illustrating cell size distribution in the sample.

The tool plots cell size diameter distribution (*Size Microns* on the *X* axis) against the *Percentage of Total Count* (*Percent* on the *Y* axis), with the scales of each axis determined by count results.



The coloring of bars reflects the nature of cells similar to how the graphic overlay is used to highlight counted cells (e.g., if testing for viability, *green* outlines live cells while *red* outlines dead cells). Other features of the histogram such as bar width, *X/Y* axis scales and number of bins used in the graph can be defined by clicking the **Customize** button. To view different aspects of the data, switch between the **Data Table**, **Histogram** and **Gates** buttons.

In addition, you can perform the following tasks:


1. To overlay data from multiple samples into a single histogram, click the **Load** button and navigate to additional diameter files.
2. Click the **Copy** button to copy the graph to your Windows clipboard, the **Print** button to send it to a printer on the network or the **Save** button to save histogram data as a file.

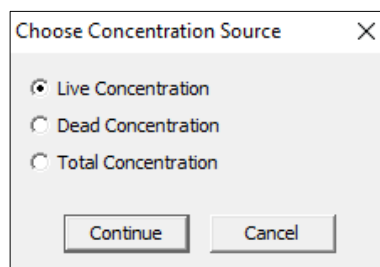
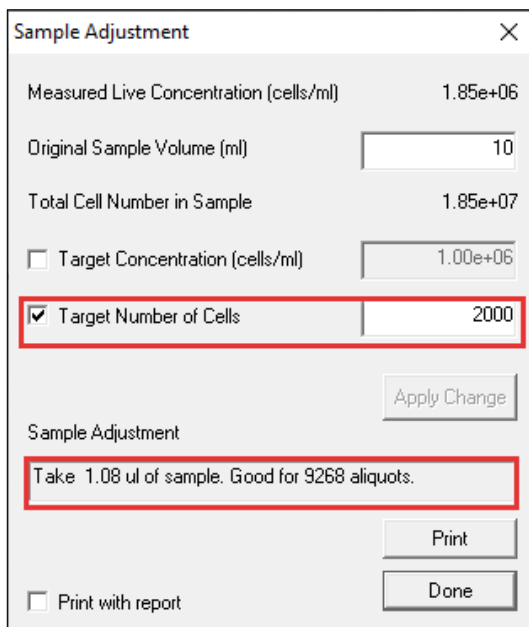
Alternatively, this tool can be activated by selecting the *Cell Size Analysis* option from the Analysis Menu.

## CALCULATE SAMPLE ADJUSTMENT

The *Sample Adjustment* tool assists you in calculating the adjustment necessary in order to achieve a specified target concentration or target number of cells in a tube. Before calculating this adjustment, you must first perform a count on either a live image or saved image to display the current cell count and concentration (or live/dead count, total cell count, viability %, live cell concentration and total cell concentration if **Test Viability** was selected).

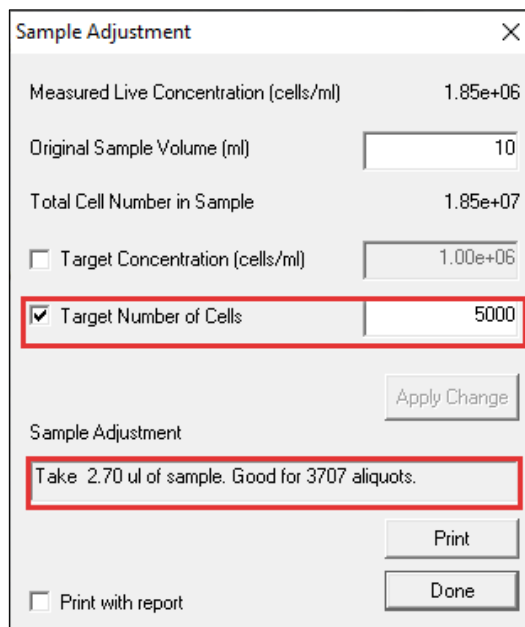
To calculate sample adjustment:

1. Click on the **Sample Adjustment** icon  to launch the tool.
2. Choose the concentration source to be used for calculating the adjustment (live, dead or total concentration) and click **Continue**.
3. Enter the **Original Sample Volume**. The *Total Cell Number in Sample* value will be updated according to sample volume.
4. Choose whether you want a **Target Concentration (cells/mL)** or a **Target Number of Cells** and enter the target concentration or cell count accordingly.
5. Click **Apply Change**. In the *Sample Adjustment* field, a statement will appear indicating how to adjust the sample to reach the targeted concentration or cell count.
6. Click the **Print with Report** check box to include adjustment results in the data report.

A screenshot of the "Sample Adjustment" dialog box. It displays the following fields and values:

- Measured Live Concentration (cells/ml): 1.85e+06
- Original Sample Volume (ml): 10
- Total Cell Number in Sample: 1.85e+07
- ☐ Target Concentration (cells/ml): 1.00e+06
- ☒ Target Number of Cells: 2000
- Buttons: "Apply Change", "Print", "Done", and "Print with report" (unchecked).
- Sample Adjustment text: "Take 1.08 ul of sample. Good for 9268 aliquots."



A screenshot of the "Sample Adjustment" dialog box, similar to the left one but with different values:

- Measured Live Concentration (cells/ml): 1.85e+06
- Original Sample Volume (ml): 10
- Total Cell Number in Sample: 1.85e+07
- ☐ Target Concentration (cells/ml): 1.00e+06
- ☒ Target Number of Cells: 5000
- Buttons: "Apply Change", "Print", "Done", and "Print with report" (unchecked).
- Sample Adjustment text: "Take 2.70 ul of sample. Good for 3707 aliquots."

Alternatively, this tool can be activated by selecting the *Sample Adjustment* option from the Analysis Menu.

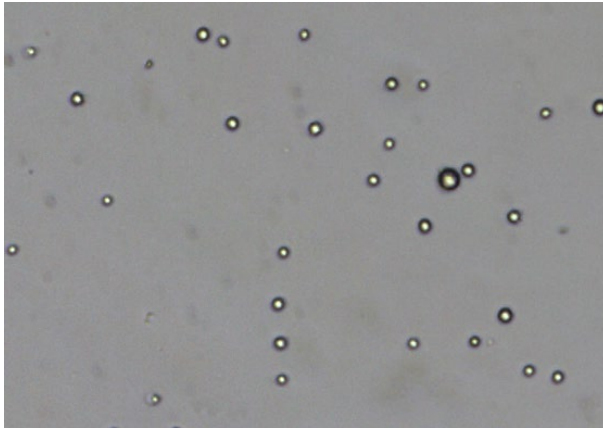
## REFERENCE OF CELL SAMPLE IMAGES

Common cell type and bead sample images taken by the Auto T4 are presented for your reference below.

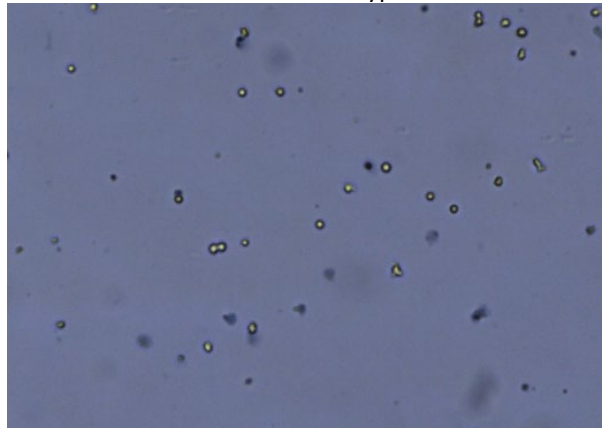
Visit the [Cellometer Auto T4](#) page on our website, click the **Applications** tab and scroll down to find additional cell type sample images.

### Cell Type Sample Images

Jurkat Cells



CD4 cells stained with Trypan Blue

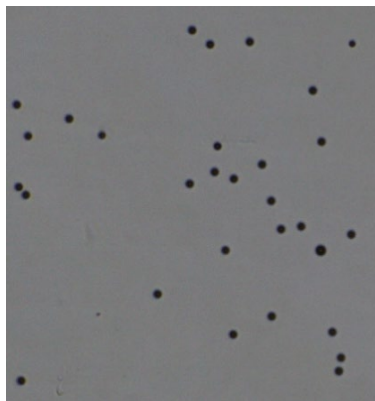


### Bead Sample Images

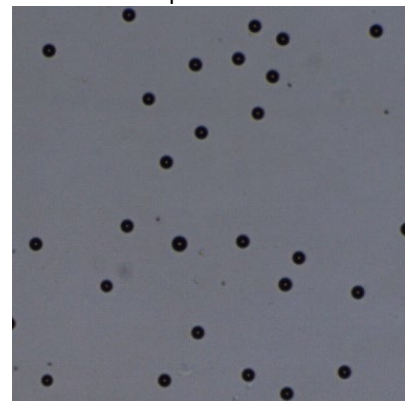
5  $\mu$ m Beads



10  $\mu$ m Beads



15  $\mu$ m Beads



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## Chapter 9. Cell Types

This chapter describes editing customizable cell type parameters to assist with declustering of clumpy cell lines. In addition, it lists all cell types included in the built-in Nexcelom Cell Library.

### EDITING CELL TYPES

Cell types can be modified to meet your requirements using parameters available on the Cell Type Screen.

#### Cell Type Screen

The screenshot shows the 'Cell Type' dialog box with the following elements and numbered callouts:

- 1** Cell Type Name: Initial Cell Type
- 2** Detailed Description: new cell type
- 3** Save as New Cell Type (checkbox)
- 4** Lock from future editing (checkbox)
- 5** Minimum Cell Diameter: 3.0 micron
- 6** Maximum Cell Diameter: 50.0 micron
- 7** Roundness: 0.10
- 8** Contrast Enhancement: 0.40
- 9** Use a default Dilution Factor of: 1.00 (checkbox)
- 10** Default Setting for "SETUP/Test Viability": No Default (dropdown)
- 11** Sensitivity: 1.0
- 12** Uniformity: 150
- 13** Very Dim Dead Cells (checkbox)
- 14** Deduster Edge Factor: 0.5
- 15** Deduster Th Factor: 1.0
- 16** Do not Deduster Clumps (checkbox)
- 17** Use Template for Printing (checkbox)
- 18** Set Default ... (button)
- 19** Browse ... (button)
- 20** Edit ... (button)
- 21** New ... (button)
- 22** Use Template for Data.Txt (checkbox)
- 23** Set Default ... (button)
- 24** Browse ... (button)
- 25** Edit ... (button)
- 26** New ... (button)
- 27** Save (button)
- 28** Print (button)
- 29** Cancel (button)

- 1 Cell Type Name** – Identifies name given to the cell type.
- 2 Detailed Description** – Contains cell type description.
- 3 Save as New Cell Type** – Saves changes to parameter settings as new cell type.
- 4 Lock from Future Editing** – Locks parameter settings so they cannot be modified.
- 5 Minimum** – Indicates minimum cell diameter to be counted.



- 6 Maximum** – Indicates maximum cell diameter to be counted.
- 7 Roundness** – Indicates minimum roundness factor to be counted.
- 8 Contrast Enhancement** – Defines contrast enhancement for cells in relation to the background.
- 9 Default Dilution Factor** – Indicates if a default dilution factor should be used and enables entry of a specific value.
- 10 Test Viability Default** – Option indicating whether Test Viability is to be used as a default.
- 11 Sensitivity** – Sensitivity value for staining of cells to be included in count.
- 12 Uniformity** – Uniformity value for staining of cells to be included in count.
- 13 Very Dim Dead Cells** – Indicates whether to include cells that do not pick up the dye well.
- 14 Decluster Edge Factor** – Decluster setting identifying how well cell edges are defined.
- 15 Decluster Th Factor** – Decluster intensity setting to be used comparing cell signals to background.
- 16 Do Not Decluster Clumps** – Indicates whether decluster enhancement settings are to be used.
- 17 Use Template for Printing** – Indicates whether to use a print template.
- 18 Set Default** – Allows users to select a default print template.
- 19 Browse** – Allows users to browse for an existing print template.
- 20 Edit** – Allows users to edit an existing print template.
- 21 New** – Allows users to create a new print template.
- 22 Use Template for Data.txt** – Indicates whether to set a data template.
- 23 Set Default** – Allows users to select a default data template.
- 24 Browse** – Allows users to browse for an existing data template.
- 25 Edit** – Allows users to edit existing data template.
- 26 New** – Allows users to create a new data template.
- 27 Save button** – Saves changes to parameter settings.
- 28 Print button** – Prints cell type parameters.
- 29 Cancel button** – Closes window without saving changes to parameter settings.

**Note:** Cell types imported from the Nexcelom Cell Library cannot be modified. However, Nexcelom cell types can be saved as a new cell type (i.e., by selecting the **Save as a New Cell Type** check box), renamed and then modified to meet your requirements.

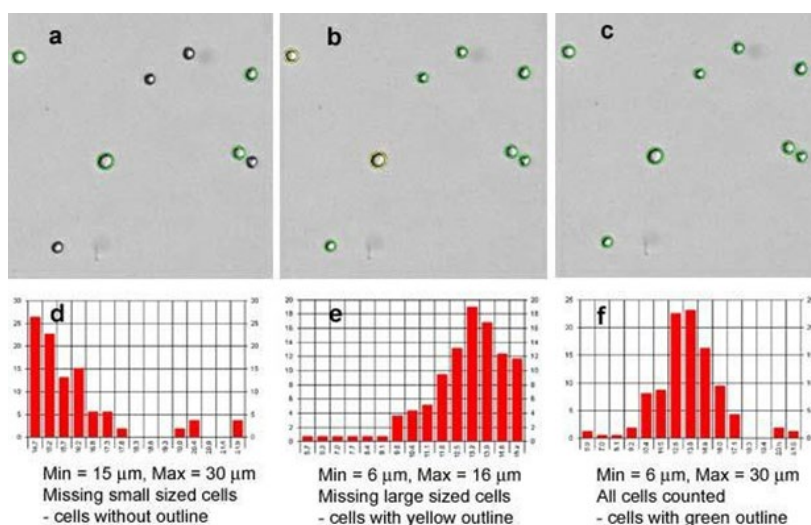


## Adjusting Cell Diameter Parameters

For each cell type, **Cell Diameter Minimum/Maximum** (in microns) parameter settings indicate the size of cells to be included in the count.

Cell Type dialog box showing parameters for 'small size cells'. The 'Cell Diameter' section is highlighted with a red box, showing Minimum: 3.0 micron and Maximum: 15.0 micron. Other parameters include Roundness: 0.30, Contrast Enhancement: 0.40, and Trypan Blue Viability Parameters.

To determine if cell diameter parameters are set up properly, use the **Counted** check box in the Home screen to display a graphical overlay outlining counted cells (e.g., green for counted cells and yellow for cells *not* counted as they are larger than the specified cell diameter). For example, the figure below shows counted cells for the same HT-29 image using different cell size parameter settings.



HT-29 cell count results were analyzed using different cell diameter parameter settings. Figure (a) – "Cell Diameter Minimum" size is too large. Cells smaller than 15  $\mu$ m are not counted, as indicated by missing outlines. Figure (b) – "Cell Diameter Maximum" is too small. Cells larger than 16  $\mu$ m are not counted, as indicated by the yellow outlines. Figure (c) – Both "Cell Diameter Minimum" and "Cell Diameter Maximum" are set up properly to include all cells. Figures (d), (e) and (f) are respective cell size histograms for (a), (b), and (c). They are also used to establish cell size parameter settings.

## Adjusting Trypan Blue Viability Parameters

Using default settings for *Trypan Blue Viability Parameters* provides the fastest counting speed for standard cell types stained a solid dark blue by trypan blue. As some cell types exhibit different morphology *after* being stained, it may be necessary to adjust parameters for accurate detection of the trypan blue population as indicated below.

### Weak blue dead cells

To pick up weak blue dead cells, select the **Very Dim Dead Cells** check box and slowly increase the **Enhancement** value to further refine the division between the background and cells with low contrast (i.e., no defined edges). *Suggested Enhancement value is 0.60; available range is 0.04 (stained cells have medium contrast to background) to 0.90 (stained cells have very low contrast to background).*

**Note:** It is a best practice to use the **Very Dim Dead Cells** check box in conjunction with other *Trypan Blue Viability Parameters* (such as *Enhancement*, *Sensitivity* or *Uniformity* values) to avoid overcounting.

Cell Type Name: small size cells

Detailed Description: small size cells

Cell Diameter: Minimum 3.0 micron, Maximum 15.0 micron

Roundness: 0.30

Contrast: 0.40

Enhancement: 0.60

☒ Very Dim Dead Cells

Trypan Blue Viability Parameters

Default Setting for "SETUP/Test Viability": No Default

Sensitivity: 1.0

Uniformity: 150

Deduster Parameters

Deduster Edge Factor: 0.5

Deduster Th Factor: 1.0

☐ Use Template for Printing

☒ Use Template for Data.Txt

Data.txt Template: <Default Template>

Buttons: Save, Print, Cancel

### Dead cells with light blue centers

To pick up dead cells with light blue centers, slowly increase the **Uniformity** value. *Suggested value is 150; available range is 100 (stained cells are all uniform in color) to 255 (stained cells have non-uniform dark and light areas).*

In addition, slowly increase the **Sensitivity** value to further refine image clarity. *Suggested value is 1.0; available range is .10 (stained cells are all dark) to 5.0 (stained cells are a mix of dark and light images).*

Variations in sensitivity values used to pick up dead cells with light blue centers are presented on the next page.

Cell Type Name: CHO-1

Detailed Description:

Cell Diameter: Minimum 8.0 micron, Maximum 38.0 micron

Roundness: 0.10

Contrast: 0.40

Enhancement: 0.60

☒ Very Dim Dead Cells

Trypan Blue Viability Parameters

Default Setting for "SETUP/Test Viability": No Default

Sensitivity: 4.0

Uniformity: 150

Deduster Parameters

Deduster Edge Factor: 0.5

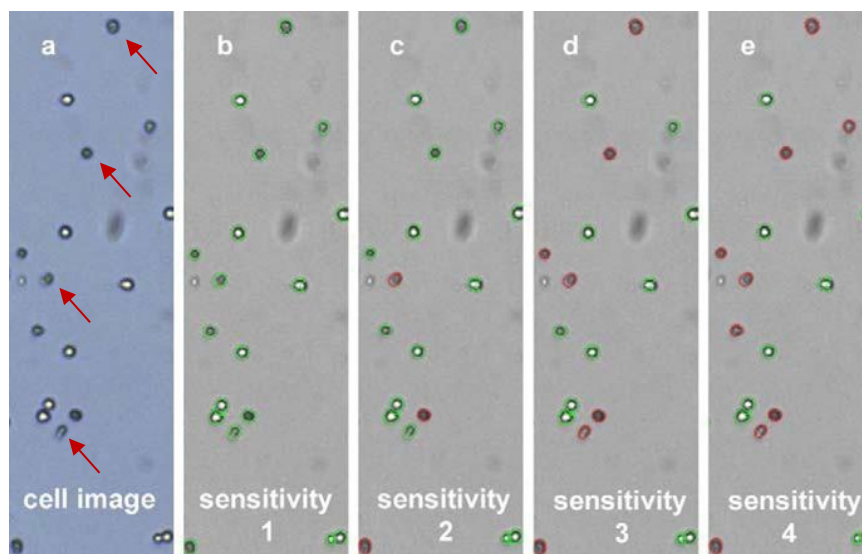
Deduster Th Factor: 1.0

☐ Use Template for Printing

☒ Use Template for Data.Txt

Data.txt Template: <Default Template>

Buttons: Save, Print, Cancel



CHO cell count results were analyzed using different "Sensitivity" settings. Figure (a) shows CHO cell image while figures (b) – (e) show an incremental increase in "Sensitivity" parameter from 1 to 4, respectively. Green outlines indicate live cells while red outlines indicate dead cells. Both live/dead cell counts in figure (e) agree with visual observation of image in (a).

## Adjusting Decluster Parameters for Clumpy Cells

The **Decluster Edge Factor** and **Decluster Th Factor** (*Decluster Threshold Factor*) parameters may be used to separate clumpy cells (i.e., connected cells for which it is difficult to distinguish clearly defined edges). *Default values for these parameters are 0.5 and 1.0, respectively.*

For the majority of cell types, the default values for these parameters are adequate to separate clumpy cells. However, some cell types (such as MCF7 and PC12) contain cells having dim boundaries within the clump. In these cases, decluster parameters can be modified to visually separate clumpy cells in the captured images.

To define edges of clumpy cells in more detail, slowly increase the **Decluster Edge Factor** and **Decluster Th Factor** values. *These parameters work in conjunction with the **Contrast Enhancement** value.*

Cell Type

Cell Type Name: MCF7\_clumpy ☐ Save as New Cell Type ☐ Lock from future editing

Detailed Description:

Cell Diameter: Minimum 7.0 micron Maximum 35.0 micron

Roundness: 0.10

Contrast Enhancement: 0.25

☐ Use a default Dilution Factor of 1.00

Trypan Blue Viability Parameters

Default Setting for "SETUP/Test Viability": No Default

Sensitivity: 1.0

Uniformity: 150

☒ Very Dim Dead Cells Enhancement: 0.60

Decluster Parameters

Decluster Edge Factor: 0.8 ☐ Do not Decluster Clumps

Decluster Th Factor: 0.8

☐ Use Template for Printing Set Default ... Browse ... Edit ... New ...

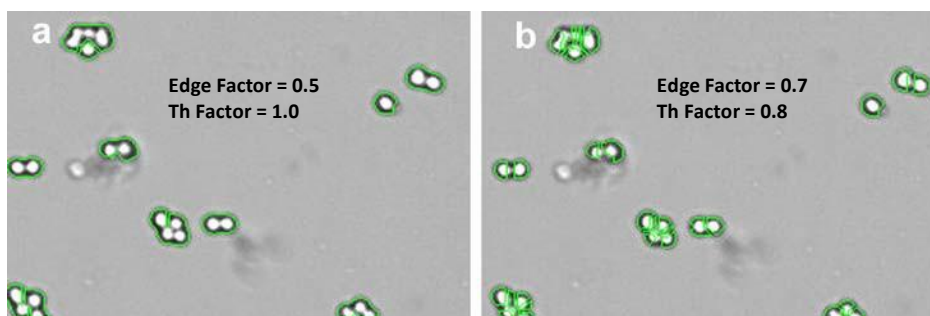
☒ Use Template for Data.Txt Set Default ... Browse ... Edit ... New ...

Data.txt Template: <Default Template>

Save Print Cancel

### SAMPLE CLUMPY MCF7 CELLS

In the sample below, clumpy MCF7 cells are analyzed using both default and modified **Deccluster Edge Factor** and **Deccluster Th Factor** parameter settings.



MCF7 cell count results were analyzed using different "Deccluster" parameter settings. Figure (a) shows default decluster settings (cells within clumps are not fully separated), resulting in MCF7 concentration of  $6.5 \times 10^5$  cell/mL. When modified declustering settings are used as shown in figure b, cells within the clumps are separated resulting in MCF7 concentration of  $8.3 \times 10^5$  cell/mL.

### DISABLING DECLUSTER FOR SMALL CELLS

Examples of small size cells are mouse splenocytes, lymphocytes, thymocytes, and PBMCs. Their cell diameters are typically less than  $7 \mu\text{m}$ .

The Auto T4's focus position for really small cells may influence cell counting when the *Deccluster* feature is enabled (i.e., the **Do not Deccluster Clumps** check box is *not* selected). *This feature works in conjunction with the Contrast Enhancement value.*

For the purpose of the following example, cell type parameter settings as shown in this screen were used to count mouse thymocyte cells in low concentration (i.e., cell counts less than 1300). Images explaining count results for various scenarios are presented.

Cell Type

Cell Type Name: small size cells ☐ Save as New Cell Type

Detailed Description: small size cells ☐ Lock from future editing

Cell Diameter: Minimum 3.0 micron Maximum 15.0 micron

Roundness: 0.30

Contrast Enhancement: 0.40

☐ Use a default Dilution Factor of 1.00

Trypan Blue Viability Parameters

Default Setting for "SETUP/Test Viability": No Default

Sensitivity: 1.0

Uniformity: 150

☐ Very Dim Dead Cells

Deccluster Parameters

Deccluster Edge Factor: 0.5

Deccluster Th Factor: 1.0

☒ Do not Deccluster Clumps

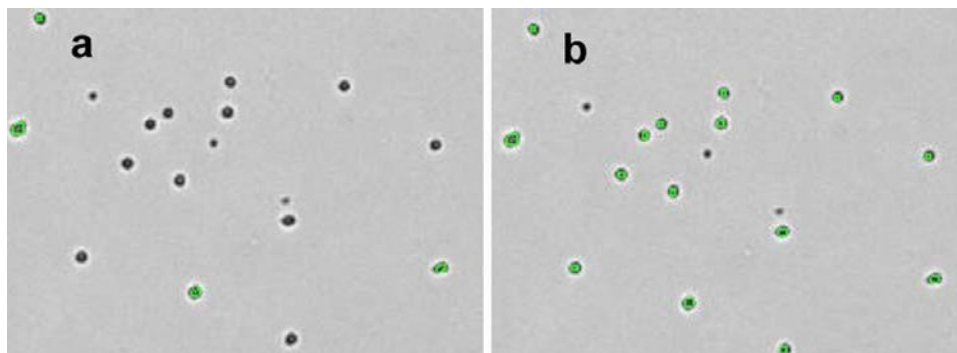
☐ Use Template for Printing Set Default ... Browse ... Edit ... New ...

☒ Use Template for Data.Txt Set Default ... Browse ... Edit ... New ...

Data.txt Template: <Default Template>

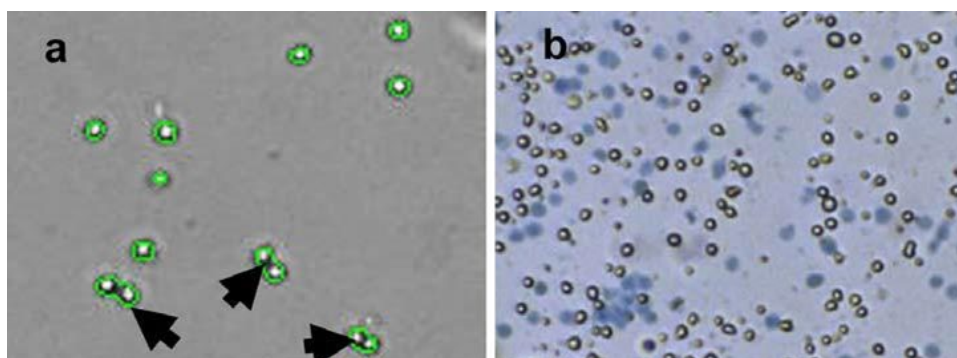
Save Print Cancel

Mouse thymocyte count results using the parameter settings identified in the screen above are shown in figure (a) with the *Decluster* feature activated (i.e., **Do not Decluster Clumps** check box is *not* selected) and in figure (b) without the *Decluster* feature. The green outlines indicate counted cells. When declustering is turned off by selecting the **Do not Decluster Clumps** check box, more cells are counted.



Mouse thymocyte count results, (a) with *Decluster* activated and (b) without *Decluster*.

However, some samples contain clumpy cells, as shown in figure (a) below. Other samples have a very high cell concentration as shown in figure (b). For these samples, the *Decluster* feature is activated (i.e., **Do not Decluster Clumps** check box is *not* selected) and the **Contrast Enhancement** value was reduced (to a value of .70).



Cell type parameters for high concentration or clumpy small-sized cells.

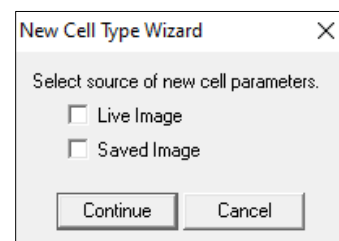
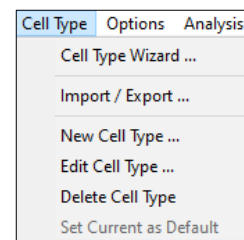
## CREATING NEW CELL TYPES

### Using Cell Type Wizard

1. Open the Cell Type menu from the menu bar and select the *Cell Type Wizard...* option.
2. Select the source of new cell parameters: **Live Image** or **Saved Image**
3. Click **Continue**.

**Note:** *Live Image* is used when Auto T4 is loaded with a counting chamber containing cells. *Saved Image* uses saved Auto T4 cell images.

4. Input a new **Cell Type Name** (replacing default entry of *new cell type*).
5. Process cell images to establish cell type parameters and click **Save**.
6. Perform a visual inspection to confirm accuracy of parameter settings.
7. Click the **Count** button.
8. Using the *View Image* control, click through views 1 to 4.
9. Visually inspect cell images and enable **Counted** check box to display overlay of green, red and yellow outlines.
10. Adjust cell type parameter settings and recount cells in the sample as necessary. See [Editing Cell Types](#) on page 41 for details.



### Creating Cell Type Manually

1. Select a cell type similar to the cells being used or choose the *Initial Cell Type* option from the drop-down.
2. Open the Cell Type menu from the menu bar and select the *Edit Cell Type...* option.
3. Check the **Save as New Cell Type** check box.
4. Modify the cell type name (by adding your initials or specify defining characteristics).
5. Process cell images to establish cell type parameters and click **Save**.
6. Click **Count** button.
7. Using the *View Image* control, click through views 1 to 4.
8. Visually inspect cell images and enable **Counted** check box to display overlay of green, red and yellow outlines.
9. Adjust cell type parameter settings and recount cells in the sample as necessary. See [Editing Cell Types](#) on page 41 for details.

## NEXCELOM CELL LIBRARY

The complete collection of cell types available for import from the Nexcelom Cell Library is listed below.

2372	DU145	HUVEC	MDA-MB-435	Raji
2H3	DU145 G37	Hybridoma	MDA-MB-468	Ramos
3T3	EL4	I9.2	MDBK	RAW
786-O	Fao	Initial Cell Type	medium size cells	RCC45
A204	Fibroblast_human	Ink-4A	MEF	RCS
A2058	Fibroblast_human_high density	INS	Melan A	RD
A3R5	Fibroblast_pig	Jurkat	Melan-3m	RD cell
A549	FRhK4	K1	Melanocyte	ReN
Alex cell	FU5AH	K562	Melanoma	RPMI7951
AR40	G37	K9 fibroblast	Mel-STR	RPMI-7951
ARPE-19	GHOST	KAT4	Mel-STV	SaOS
Astrocyte	GM cell	KR158	MES-SA	SF9
aTC6	GRANTA	KT1	MG63	SK28
B16	H157	KT2	MM1S	SK-MEL-28
BHK	H358	KU812	MMS	SKOV3
BT	H42E	L cell	MNT1	small size cells
BXPC3	H520	L1210	Mouse ES feeder cell	SNU475
C2C12	H647	L929	MRC5	SW48
C6	H9	large size cells	MSB1	SW480
C6-36	HASMC	LCL	MTA-9	SW620
CALU6	HBL-2	LLC-PK1	MV411	TH1
CCR5	HC9	Lncap	N59	THP
CEF	HCE4	Lymphoma	NCI-460	Til-T
CGN	HCT15	M19	NCI-H929	TME-7
CHO-1	HDF	Macrophages	ND468	Tn5
CL-1	HEC	MAGIC	new cell type	TS
Colo205	HEK293	Malme 3M	NIH 3T3	U118
Cos7	HEK293T	Mast	NM4A	U266
CRL-1863	Hela	MC32A	NM4A4	U2OS
CT-26	High 5	MC32A-B7	OCIM1	U87
CT-26-B7	HL-60	MC-38	OVCAR	U937
CT-26-CEA	HMN	MC-38-B7	P19	UACC 257
CT26-CEA-B7	HNPM	MCF-10A	PA-317	V12
D1.1	HOS	MCF-10A4	PC	V529
DC145	HOS MNNG	MCF7 clumpy	PC-12	Vero
Dictyoslelium	HPAF II	MCF7 non-clumpy	PC3	WIDR
DLD	HT-1080	MCF7_clumpy	PDAC	Wil2S
D-MEL	HT-29	MDA-231	PLHC	
DU	HUT78	MDA-MB-231	QNR-K22	

If you would like help creating a new cell type or customizing cell type parameters for a sample, send saved raw images to [support@nexcelom.com](mailto:support@nexcelom.com) and we will optimize a custom cell type for you. See [Saving Data and Images](#) on page 51 for details on how to save the information that Nexcelom will need.

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# Chapter 10. Data Management

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This chapter describes how to save count result data and image files, export them to an Excel spreadsheet and how to load previously-saved data and images back into the Auto T4 for reanalysis.

## SAVING DATA AND IMAGES

### Manually Saving Data

1. Perform a cell count on the sample currently being viewed.
2. Click the **Select Data File** icon  in the bottom left corner of the screen to navigate to a folder on the Operating Computer or network where the data file is to be saved.
3. Enter a name for the data file (e.g., *data*) and click **Open** to return to the Home screen.
4. To save data (i.e., cell count and parameter settings associated with the count), click the **Save Data** button.
5. Click **OK** in response to the *Save Data* confirmation prompt. *Data is saved to a text (.txt) file.*

**Note:** Count results are saved by default to a single .txt file in which rows (logged with a date/time stamp and Sample ID) are appended each time data is saved. Rows include key parameter settings used for the count.

6. To view your data, click the **View Saved Data** button or navigate to the folder and open the data file.

Alternatively, you can use the *New Data File* and *Open Data File* options available on the File Menu to create a data file and view saved data.

### Manually Saving Images

1. Perform a cell count on the sample currently being viewed.
2. Open the File Menu and select either the *Save Images* or *Save Counted Images* option. Choosing *Save Counted Images* includes the graphic overlays that were created during the counting process.
3. Navigate to a folder on the Operating Computer or network where image files are to be saved.
4. Enter a name for the images file folder to be created and click **Save** to return to the Home screen.
5. Click **OK** in response to the *Save Images* confirmation prompt.

## Automatically Saving Data

1. Open the Options Menu and select the *Save Options...* option.
2. Click the **Auto Save data.txt** check box to select it.
3. If you want to create a new data text file each time cells are counted, click the **Create new data.txt with each sample check** box to select it. *Keep in mind that selecting this option may result in a large volume of files.*  
**Note:** If this option is *not* selected, a new row containing a date/time stamp and key parameter settings will be appended to the existing data file each time cells are counted.
4. Click the **Set Folder** button to navigate to a folder on the Operating Computer or network where data files are to be saved. *It is recommended that data files be saved to a folder on the desktop or USB drive.*
5. Enter a file name (e.g., *data*) and click **Open** to return to the Home screen.

Once auto saving of data is set up, results will be saved to the specified folder and file each time cells are counted.

## Automatically Saving Images

1. Open the Options Menu and select the *Save Options...* option.
2. Click the **Auto Save Sample Image** check box to select it.
3. 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).  
**Note:** Nexcelom Support will require raw images when requesting assistance with optimization of cell type parameters or troubleshooting.
4. Click the **Set Folder** button to navigate to a folder on the Operating Computer or network where image files are to be saved. *It is recommended that image files be saved to a folder on the desktop.*
5. Enter a file name (e.g., *SampleImage*) and click **Open** to return to the Home screen.

Once auto saving of images is set up, files will be saved to the specified folder each time cells are counted.

## LOADING DATA AND IMAGES


### Loading Data Files

1. Open the File Menu and select the Open Data File option.
2. Navigate to a folder on the Operating Computer or network where data files are stored and select data file to be loaded. *Data file selected should contain data previously saved from the instrument in a .csv format.*
3. Click **Open** to load the data and return to the Home screen.
4. Click **View Saved Data**.

### Loading Image Files

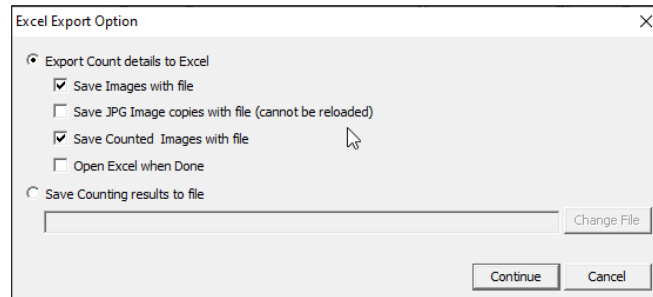
1. Open the File Menu and select the *Load Image for Display* option (to load image for viewing) or *Load Image and Count* option (to load image and perform a count).
2. Select the image file to load and click **Open** to return to the Home screen.

**Note:** If the **Intelligent File Load** check box in the *Counting Options* screen (available on the Options Menu) is selected, you will only need to select a file that ends in “-A” as the remaining image will load automatically. Otherwise, the File Explorer window will open again for you to select the associated “-B” image.

In addition, if the *Load Image for Display* option was selected, only saved images will be displayed for viewing. When software is in *Data Analysis Mode*, the **Count** button will *not* be enabled. Click the **Recount** icon  to re-analyze the captured image.

## EXPORTING DATA AND IMAGES

Data and images can be exported by clicking the **Export to Excel** icon  after performing a cell count.



1. To **Export Count Details to Excel**, choose items to be included in the export (e.g., images with data file, JPG image copies with file, counted images with file) and indicate whether to open **Excel when Done**. Click **Continue**. When prompted, navigate to a folder on the Operating Computer or network where data files are stored, enter a file name and click **Save**. *Data is saved as an .xlsx file and associated images (if selected for export) will be stored in a folder using the same name.*
2. To **Save Counting Results to File**, click the **Change File** button to navigate to a folder on the Operating Computer or network where data files are stored, enter a file name and click **Open**. Click **Continue** to save the file and return to the Home screen. *Data is saved as an .xlsx file.*

Alternatively, you can use the *Export Results to Excel* option available on the File Menu to export data and images.

# Chapter 11. Cleaning, Maintenance and Storage

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Keeping the Auto T4 and its operative area clean between runs, during use and post runs is a best practice and prevents contamination. Caring for the instrument and its consumables is also a best practice.



**CAUTION:** Always power OFF the instrument before cleaning as damage to the machine could occur.



**CAUTION:** Allow for flammable agents used for cleaning or disinfecting (or as solvents for adhesives) to completely evaporate before powering ON the instrument.

## CLEANING

The instrument and any cords/cables can be wiped down using a 70% Isopropyl (IPA) solution. Repeat until the soil is no longer visible. Finish with a fiber optic lint-free wipe (e.g., Kimwipes).

1. Dampen a fiber optic lint-free wipe with IPA.
2. Use the wipe to rub lightly on the outside of the instrument until it is visibly clean.
3. Wait for the cleaning agent to evaporate before powering the instrument ON.

Should something break or be spilled inside the device, power OFF the instrument and contact Nexcelom Support at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

## ROUTINE MAINTENANCE

No one other than Nexcelom-authorized personnel may service inside the protective instrument cover of the Auto T4. Contact Nexcelom Support or an authorized service representative to address any changes in instrument output or performance.



**WARNING:** Do not remove the instrument cover due to an electric shock hazard. Contact Nexcelom Support for assistance.

### Contacting Nexcelom Support

All technical questions regarding maintenance should be directed to Nexcelom Support at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

## Preventive Inspection and Maintenance

Regular preventive inspections should be carried out to reduce safety concerns of the instrument due to aging, normal wear and tear, etc. The manufacturer assumes no responsibility for improper changes or repairs carried out on the instrument or its accessories by unauthorized persons. The warranty will immediately become void should an unauthorized personnel attempt to in any way repair or modify the instrument.

Contact Nexcelom Support to schedule all preventive maintenance needs and address any functionality concerns at +1 (978) 327-5340 or via email: [support@nexcelom.com](mailto:support@nexcelom.com)

## STORAGE

When preparing the instrument for storage:

- Always thoroughly clean the instrument, cables and any of the accessories or consumables before storage.
- Check for any damage and if possible, re-package the Instrument and Operating Computer.
- Ensure that storage temperature and spatial requirements are met (see [Site Preparation](#) on page 14).
- DO NOT put anything heavier than  $\geq 25$  lbs. on the instrument or the box in which it is stored.

Store the cables neatly, checking for any signs of damage or wear frequently and immediately before/after use. Do not allow the cables to become kinked or tangled. Do not set heavy objects on any of the accessories or consumables.



Always store beads and reagents according to their information for use documentation.

# Chapter 12. Troubleshooting and FAQs

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This chapter lists troubleshooting steps for resolving potential issues, common software error messages and *Frequently Asked Questions* (FAQs).

## TROUBLESHOOTING AND ERROR MESSAGES

### Particles appear in the background/background is too dark

- Take a new background image by following the steps presented in [Taking a Background Image](#) on page 18.

### Instrument cannot be powered on

- Check to ensure the Power Cord that was provided with the instrument is being used.
- Check Power Cord to confirm it is not kinked or tangled.
- Check that Power Cord is plugged in properly to both the outlet and the instrument.
- Check that Power Switch on instrument is set to the ON position.

### Instrument displays software error messages

The Auto T4 software has built-in error messaging when functionality is operating improperly. A few common error messages are listed below:

- *No instrument detected.*
- *Background was saved, but .... Dim XXX counts*
- *Error setting up Preview*
- *Could not read GPIO Values*
- *Running software in data analysis mode.*

#### IF ERROR REQUIRES REBOOTING THE INSTRUMENT

If error encountered cannot easily be resolved, follow the steps listed below to reboot the Auto T4 system and repeat the task to be performed.

1. Close out of the software.
2. Shut down and restart the Operating Computer.
3. Power OFF the instrument.
4. After the Operating Computer has restarted, power ON the instrument.
5. Restart the Auto T4 software.



If the problem persists, contact Nexcelom Support for assistance at +1 (978) 327-5340 or email: [support@nexcelom.com](mailto:support@nexcelom.com)

*IF ERROR REQUIRES CREATING A SUPPORT TICKET*

If error encountered that *cannot* be resolved and/or the problem persists after rebooting the Auto T4 system, perform the following steps before creating a Support ticket:

1. Record the error message.
2. Record the sequence of events that caused the error, if possible.
3. Close the error message window.



This information may be helpful to Nexcelom Support when troubleshooting the issue. Contact Nexcelom Support for assistance at +1 (978) 327-5340 or email: [support@nexcelom.com](mailto:support@nexcelom.com)

## FREQUENTLY ASKED QUESTIONS

### Can SD025 and SD300 slides be used on the Auto T4?

No, the Auto T4 is designed to be used *only* with the SD100 slides.

### How do I upgrade to the newest version of the software?

Contact [support@nexcelom.com](mailto:support@nexcelom.com) with the serial number of the instrument to request an upgrade.

### The Power Cord or USB Connector Cable for the instrument has been lost.

Contact [support@nexcelom.com](mailto:support@nexcelom.com) with the serial number of the instrument to request replacements.

### The cell type needed does not appear in the Cell Type drop-down.

Cell types can be imported from either the Nexcelom Cell Library or from custom libraries stored in external network locations. See [Importing Cell Types](#) on page 26 for instructions on how to import a cell library and populate the Cell Type drop-down menu with selected cell types.

### When testing for cell viability, count results are lower than expected.

When preparing a sample to test for cell viability, ensure that the trypan blue stock concentration used is 0.2%. If stock concentration is 0.4%, it is recommended you dilute it with a balanced salt buffer (e.g., PBS) and filter diluted solution with a 0.2 micron filter before adding stain to your sample. Using trypan blue in a concentration higher than 0.2% will make the cells more difficult to detect and may result in counting inaccuracies.



# Chapter 13. Nexcelom Support

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This chapter presents the scope of available Nexcelom Support services and provides global contact information.

## SCOPE OF SUPPORT SERVICES

Nexcelom is dedicated to providing our customers with outstanding support including the following services:

- Online and in-lab customer training
- Creation of new cell types
- Optimization of counting parameters
- Troubleshooting via telephone
- Periodic safety checks and functional evaluations (offered as part of a separate maintenance contract)

To explore Nexcelom Support, training and resources available online or in your area, visit our website at [Nexcelom.com/training-and-support/](http://Nexcelom.com/training-and-support/) or contact Nexcelom Support.

## CONTACTING SUPPORT

If there is a technical problem with your instrument or software, contact Nexcelom Support.

### Nexcelom Corporate Headquarters

Nexcelom Bioscience  
360 Merrimack Street, Suite 200  
Lawrence, Massachusetts 01843  
USA

Phone: (978) 327-5340

Fax: (978) 327-5341

[support@nexcelom.com](mailto:support@nexcelom.com)

### San Diego Office

Nexcelom Bioscience LLC  
11100 Roselle Street, Suite B  
San Diego, California 92121  
USA

Phone: (978) 327-5340

Fax: (978) 327-5341

[support@nexcelom.com](mailto:support@nexcelom.com)

### Manchester Office

Nexcelom Bioscience Ltd  
Unit 5, Rutherford House  
Pencroft Way  
Manchester Science Park  
Manchester  
M15 6SZ  
United Kingdom

Phone: +44 (0) 161 232 4593

[support@nexcelom.com](mailto:support@nexcelom.com)

### Shanghai Office

Nexcelom Bioscience Instruments  
(Shanghai) Co. Ltd  
Unit 4-E, North Building  
No.2966, Jinke Rd,  
Pudong New District, Shanghai  
201203  
China

Phone: (+86) 21 5886 0038

[support@nexcelom.com.cn](mailto:support@nexcelom.com.cn)

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## Appendix A. Consumables

This appendix presents Nexcelom consumables designed specifically for the Auto T4 such as disposable chambered slides, beads and reagents. Catalog numbers are listed for all available sizes.

### DISPOSABLE CHAMBERED SLIDES

Cellometer *Cell Counting Chambered Slides* are compatible with all Cellometer Systems. Each all-plastic, disposable slide contains two sample counting chambers with a precisely-controlled height. The fixed 20µl sample size allows for simple, automated calculation of cell concentration following imaging and counting. Image-based counting with disposable counting chambers offers several key advantages:

- No potential clogging
- Ideal for fragile samples, such as hepatocytes
- No washing
- No potential cross-contamination




Catalog #	Description	Size	Unit	Image
CHT4-PD100-002	Standard chamber thickness. Packed in microscope slide boxes. Individually QC-Checked. 4-year expiration.	Box of 100 slides for 200 counts	1 Box	
CHT4-PD100-003	Standard chamber thickness. Packed in microscope slide boxes. Individually QC-Checked. 4-year expiration.	Case of 500 slides for 1,000 counts (10 individual boxes)	1 Case	
CHT4-PD100-303	Standard chamber thickness. Packed in microscope slide boxes. Individually QC-Checked. 4-year expiration.	Case of 1,500 slides for 3,000 counts (30 individual boxes)	3 Cases	
CHT4-PD100-503	Standard chamber thickness. Packed in microscope slide boxes. Individually QC-Checked. 4-year expiration.	Case of 2,500 slides for 5,000 counts (50 individual boxes)	5 Cases	

Catalog #	Description	Size	Unit	Image
CHT4-SD100-002	Standard chamber thickness. Packed with protective film. 1-year expiration.	Box of 75 slides Good for 150 counts	1 Box	
CHT4-SD100-014	Standard chamber thickness. Packed with protective film. 1-year expiration.	Case of 900 slides Good for 1,800 counts	1 Case	
CHT4-SD100-314	Standard chamber thickness. Packed with protective film. 1-year expiration.	Case of 2,700 slides Good for 5,400 counts	3 Cases	
CHT4-SD100-514	Standard chamber thickness. Packed with protective film. 1-year expiration.	Case of 4,500 slides Good for 9,000 counts	5 Cases	

Visit the [Nexcelom-products/cellometer-disposable-counting-chambers](https://www.nexcelom.com/products/cellometer-disposable-counting-chambers) page on our website for an up-to-date list and to purchase Cellometer Counting Chambered slides directly from Nexcelom Bioscience.

## BEADS AND REAGENTS

Nexcelom Bioscience provides a wide variety of beads and reagents for all of your analysis needs.

Catalog #	Description	Size	Unit	Image
B05-02-050	5 $\mu$ m polystyrene bead in Trypan Blue Solution. Good for 200 counts	1 mL	4 Vials	
B10-02-020	10 $\mu$ m polystyrene bead in Trypan Blue Solution. Good for 200 counts	1 mL	4 Vials	
B15-02-010	15 $\mu$ m Polystyrene bead in Trypan Blue Solution. Good for 200 counts	1 mL	4 Vials	

Visit the [Nexcelom-products/reagents](https://www.nexcelom.com/products/reagents) page on our website for an up-to-date list and to purchase reagents for use with Cellometer instruments directly from Nexcelom Bioscience.

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# Appendix B. Declaration of Conformity

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## According to ES3043-1

Manufacturer: Nexcelom Bioscience LLC  
Address: 360 Merrimack Street, Suite 200, Lawrence MA 01843 USA  
Product Name: Cellometer Auto T4 High-Speed Cell Counter      Model Number: Cellometer Auto T4

Conforms to the appropriate country standards and governing regulations listed below. We, as the manufacturer, are fully responsible for the design and production of the above-mentioned equipment.

### Safety:

- EN 61010-1:2010 Safety requirements for electrical equipment for measurement, control, and laboratory use

### EMC:

- EN 55032:2012/AC:2013 Class A ITE emissions requirements (EU)
- ICES-003 Issue 6 Class A Digital Apparatus emissions requirements (Canada)
- CFR 47 FCC Part 15 Subpart B Class A emissions requirements (USA)
- AS/NZS CISPR 32:2013 Class A ITE emissions requirements (Australia)
- VCCI-CISPR 32:2016 Class A ITE emissions requirements (Japan)
- EN 61000-3-2:2014 Limits for harmonic current emissions (equipment input current up to and including 16A per phase)
- EN 61000-3-3:2013 Limitation of voltage fluctuations and flicker in low voltage supply systems for equipment with rated current up to and including 16 A
- EN61326-1:2013 EMC requirements for Electrical equipment for measurement, control and laboratory use - General Use

### Supplementary Information:

The product herewith complies with the requirements of the Low Voltage Equipment Directive 2014/35/EU and the EMC Directive 2014/30/EU and carries the CE marking accordingly.

This product was tested in a typical configuration.

This device complies with Part 15 of the FCC rules. Operation is subject to the following two conditions: 1) this device may not cause harmful interference, and 2) this device must accept any interference received, including interference that may cause undesired operation.



Matthew Bularzik  
Head of Quality

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# Appendix C. Warranty and License Details

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This appendix presents *Warranty Information* for Cellometer instruments, Nexcelom's *Limitation of Liability (Hardware and Software)* statement, and *Terms and Conditions* related to the use of Cellometer software and related documentation. In addition, it includes a definition of *Nexcelom's Proprietary Information*.

## WARRANTY INFORMATION

Nexcelom warrants that Cellometer instrumentation products shall, for a period of twelve (12) 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 manufacturer's 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.

Instruments must be handled and packaged correctly when shipping to other locations. Contact Nexcelom for additional information and to order packaging materials.

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.

## TERMS AND CONDITIONS

The *Nexcelom Bioscience LLC – Terms and Conditions of Sale* license agreement states the terms and conditions upon which Nexcelom Bioscience offers to license to you the software together with all related documentation. The Software is licensed to you for use only in conjunction with Nexcelom's family of products.

In addition, the original Cellometer software and any software upgrades installed on your Cellometer system by authorized representatives of Nexcelom Bioscience LLC is protected. You may not tamper with this software (including unauthorized upgrades), disclose it to third parties or use it for any purpose other than running your Cellometer system. Nexcelom Bioscience LLC does not grant you any other rights to use or disclose the original Cellometer software or upgrades, and any further uses will be prosecuted by Nexcelom Bioscience LLC to the maximum extent possible by law. Any other use of Cellometer software or upgrades is explicitly prohibited. In addition, you may not disclose Cellometer software, upgrades, or any of its features and benefits to a third party.

### Nexcelom Proprietary Information

Cellometer products have been developed by Nexcelom Bioscience LLC and include certain intellectual property of Nexcelom, including without limitation, software, samples, schematics, specifications, manuals, designs, and other technical, business, trade secret, proprietary and confidential information provided to Buyer by Nexcelom ("Nexcelom Proprietary Information").

Buyer is granted a non-exclusive right and license to use the Nexcelom Proprietary Information solely: (a) as incorporated into, and in conjunction with, the products, (b) in conformance with the specifications, and (c) for Buyer's internal use.

Buyer may not: (i) assign, sublicense, transfer, lease, rent or distribute any of its rights in the Nexcelom Proprietary Information; (ii) port, translate, localize or create derivative works based upon the Nexcelom Proprietary Information in any manner; (iii) reverse assemble, decompile, reverse engineer, translate or otherwise attempt to derive or obtain the source code, the underlying ideas, algorithms, structure or organization of the Nexcelom Proprietary Information; (iv) use the Nexcelom Proprietary Information for the benefit of any third party including as part of any service bureau, time sharing or third party training arrangement; or (v) publish any benchmark testing results on any product or the Nexcelom Proprietary Information without Nexcelom's written consent.

Nexcelom Bioscience LLC retains all ownership rights in the Nexcelom Proprietary Information and, other than limited license set forth in this section, Buyer shall have no right in or to the Nexcelom Proprietary Information.

Buyer will not disclose the Nexcelom Proprietary Information to any third party or use it in any manner outside the scope of the license including: (1) developing, designing, manufacturing, engineering, reverse engineering, refurbishing, selling or offering for sale items, parts or components of items, derivatives of or equivalents, or (2) assisting any third party in any manner to perform such activity.

Buyer shall use reasonable care to protect the Nexcelom Proprietary Information, and in no event less than the care Buyer uses to protect its own like information.

## LIMITATION OF LIABILITY (HARDWARE AND SOFTWARE)

Cellometer® automated cell counting instruments, software and consumables are intended for research use only.

In no event shall Nexcelom be liable for any damages whatsoever (including, without limitation, incidental, direct, indirect, special or consequential damages, damages for loss of business profits, business interruption, loss of business information) arising out of the use or inability to use this Software, Consumables or related Hardware.



Cellometer® Auto T4 User Manual

8001009, Rev C

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