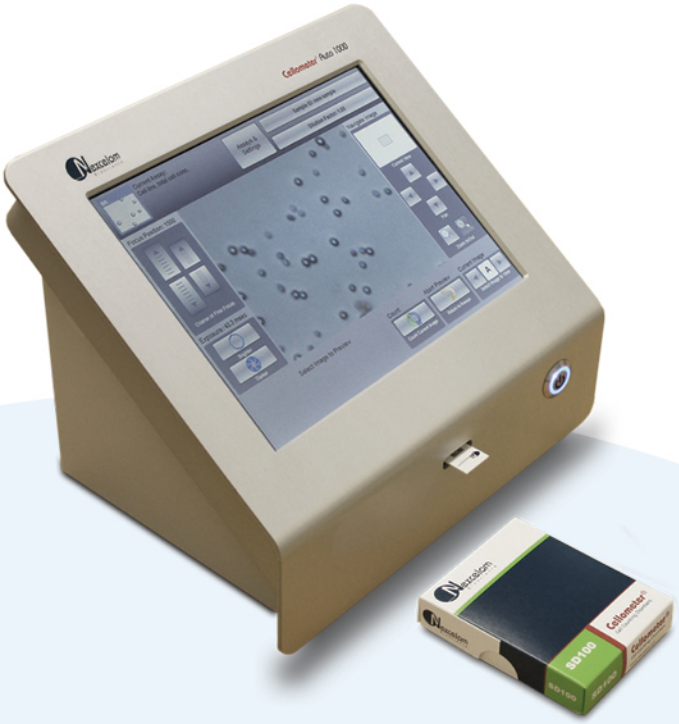
Cellometer® Auto 1000

Brightfield Cell Counter



User Manual

Nexcelom Bioscience

Cellometer® Auto 1000 User Manual

8001505, Rev D

March 2022

Table of Contents

Chapter 1. Introduction

This chapter presents introductory information to be reviewed *prior* to unpacking your Cellometer® Auto 1000 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 1000 INSTRUMENT 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 cells to be counted, users can adjust parameters based on cell morphology and perform reanalysis as necessary. Cellometer *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 1000 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 1000 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 live and dead cells with color-coded outlines in the image being viewed.

The Auto 1000 is an all-in-one standalone instrument offering a large touch screen that ensures simple operation and user-friendly visualization of cell morphology and counted cells. As the built-in computer can be attached to a network, count results and images can be stored to an external location (or on the included USB drive) and an Auto-Print feature can be enabled to generate a report for each counted sample. *Assays* allow users to customize counting parameters for specific cell types and a *Cell-size Analysis* tool generates histograms enabling optimization of cell diameter settings (e.g., to exclude debris or very large cells) during analysis of count results.

Intended Use

Intended use of the Auto 1000 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, see *Troubleshooting and Error Messages* on page *65* 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 1000 Instrument

Power Cord

USB Drive

Cellometer 1000 User Manual and **Quick Start Guide**

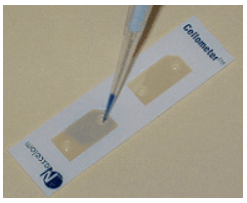
Available as .pdf files on the USB Drive

Focus Guide

Graphic sheet intended to help users adjust instrument focus

Counting Chamber Slides

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



ABOUT THIS USER MANUAL

This *Cellometer Auto 1000 User Manual* provides information on the following topics:

- Overview and Shipping Contents
- Potential Hazards and Safety Information
- Components, SN Label and System Specs
- Site Preparation and Unpacking
- Using Instrument for the First-time
- Key Software Screens
- Slide Preparation and Loading Samples
- Basic Counting and Analysis Workflow
- Assays and Customization Parameters
- Cell Types and Customization Parameters
- Defining User Counting/Saving Options
- Cleaning, Maintenance and Storage
- Troubleshooting and FAQs
- Nexcelom Support Services and Contacts
- Consumable Counting Slides and Beads
- Declaration of Conformity
- Warranty and License Details

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.**

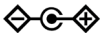
GLOSSARY OF ABBREVIATIONS

The following abbreviations may be displayed on the shipping container, on the Cellometer Auto 1000 device label or in this user manual.

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

GLOSSARY OF SYMBOLS

The following international symbols may be displayed on the shipping container, on the Cellometer Auto 1000 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.**				

Chapter 2. Equipment Safety

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 1000 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

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

Servicing Hazard

No one other than Nexcelom-authorized personnel may service inside the protective cover of the Auto 1000 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.

ENVIRONMENTAL REQUIREMENTS

Environmental requirements for the intended operation of the Auto 1000 instrument are presented below.

- For Indoor Use Only
- Elevation: 0 to 2,000 m
- Temperature Range: 10 °C to 30 °C
- Relative Humidity: 0% to 90% RH, non-condensing
- Pollution degree: Degree 2
- MAINS supply voltage fluctuations up to ± 10% of the nominal voltage

IMPORTANT: If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

SAFETY INFORMATION

Personnel operating and maintaining the Auto 1000 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 1000 instrument to the main power connection *prior* to use. The Auto 1000 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 1000 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

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.

This page intentionally left blank.

Chapter 3. Instrument Description

This chapter describes the Auto 1000 instrument components, device label and system specifications.

AUTO 1000 COMPONENTS

Auto Sample Slot: Counting chamber slides are inserted into this slot for viewing (accepts only one slide at a time).

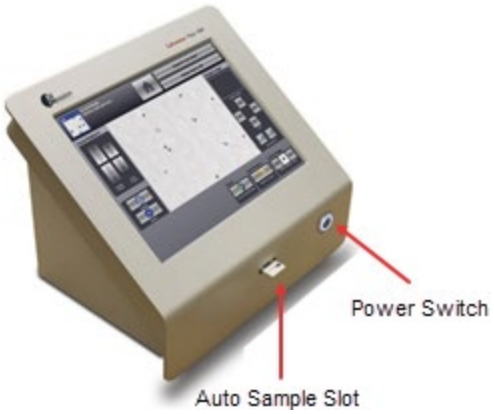
Power Switch: Controls power going to the instrument.

USB 2.0 Cable Port: Port used to connect USB 2.0 devices (e.g., printer, mouse or thumb drive).

Ethernet Port: Port used to connect a network or internet cable to access the Auto 1000.

Power Cord Connector Input: Port used to connect the power supply to the Auto 1000.

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



DEVICE LABEL

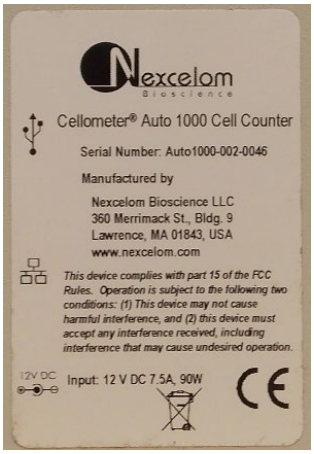
SN — Serial Number

Manufacturer

Polarity DC Power Connector

Waste Electrical and Electronic Equipment Directive

CE — Conformité Européenne 93/42/EEC



SYSTEM SPECIFICATIONS

The Auto 1000 is your all-in-one automated cell counting system. Using brightfield imaging and pattern-recognition software, it quickly and accurately identifies and counts individual cells. Results are automatically calculated and include cell counts, concentration, diameter and % viability.

	Cellometer Auto 1000 Cell Counter
Channels	Brightfield
Number of Channels	1
Commonly Used Compatible Dyes	Trypan Blue
Counting Speed Per Sample	<30sec
Volume (per chamber)	20 µL
Cell Size/Diameter Range	5 – 300* µm
Concentration Range	$1x10^5 – 1x10^7$
Software	Cellometer Auto Pattern-recognition software to quickly and accurately decluster, identify and count individual cells
Weight	9 lbs (4.1 kg)
Width	11.1” (28.2 cm)
Depth	8.3” (21.1 cm)
Height	9.0” (22.9 cm)
Input to Power Adapter	100-240 VAC, 50/60 Hz, 1.5A
Output to Instrument	12 VDC, 7.5A

*Cellometer CHT4-PD300 Slides are required for cells > 80 microns in diameter.

Chapter 4. Unpacking and Site Preparation

This chapter presents site preparation facility requirements for setting up the Auto 1000 instrument 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 your new Cellometer Auto 1000 (see *Facility Requirements* on page *12*). Visit the Cellometer Auto 1000 page on our website, click the **Resources** tab and scroll down to find available *Videos* and *Training Webinars* for this instrument.

Note: Steps for unboxing the Cellometer Auto 1000 and Auto 2000 instruments are the same and the software GUI is similar for both instruments. To view a video on unboxing and getting started, visit the Cellometer Auto 2000 page on our website, click the **Resources** tab and scroll down to find *Training Videos*.

Our staff would be happy to help you set up your Auto 1000. Contact Nexcelom Support for assistance at +1 (978) 327-5340 or via email: 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 and USB Thumb Drive.
7. Remove the Auto 1000.
8. Place the Auto 1000 in the prepared area (see *Facility Requirements* on page *12*).
9. Remove the protective seals around the Auto 1000.
10. Insert the Power Cord Connector into the Auto 1000.
11. Plug the Power Cord into a surge protector (recommended) or power outlet.
12. Turn the Auto 1000 Power Switch to the ON position. The system is now ready for use.

Auto 1000 IQ/OQ Validation

Nexcelom Bioscience has designed an *Installation Qualification/Operation Qualification (IQ/OQ)* validation specifically for the Cellometer Auto 1000. 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

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 1000.

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

Keep the area around the Auto 1000 clean between, during and post operation.

Electrical requirements for the Auto 1000 are as follows:

- 1.5 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 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

Chapter 5. Operation

This chapter describes the steps to be performed when using the Auto 1000 instrument for the first time. As the Auto 1000 is a touch-screen device, tap gently on screen elements (e.g., icons or buttons) using a finger or stylus to make software graphical user interface (GUI) selections. *As an alternative, a USB mouse may be connected.*

WARNING: Never place a foreign object inside the Auto Sample Slot (which is designed to accept only one slide at a time). 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

USING INSTRUMENT FOR THE FIRST TIME

Starting the Software

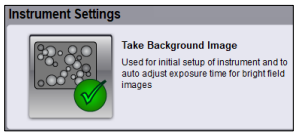
Power ON the instrument. The Auto 1000 software graphical user interface (GUI) will load automatically.

Taking a Background Image

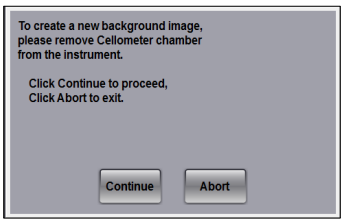
After initial setup of the instrument, you must take a background image of the system by itself (without the counting chamber slide inserted) to normalize the cell counter. Unless the instrument is moved to a new location, there is generally no need to take another background image.

1. Tap the **Settings** icon located on the bottom panel of the Home screen to edit instrument settings.

2. In the *Instrument Settings* area, tap the **Take Background Image** icon.

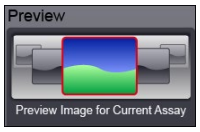


3. Remove the slide from the instrument, if applicable. Tap **Continue** in response to pop-up message to confirm Auto Sample Slot is empty.

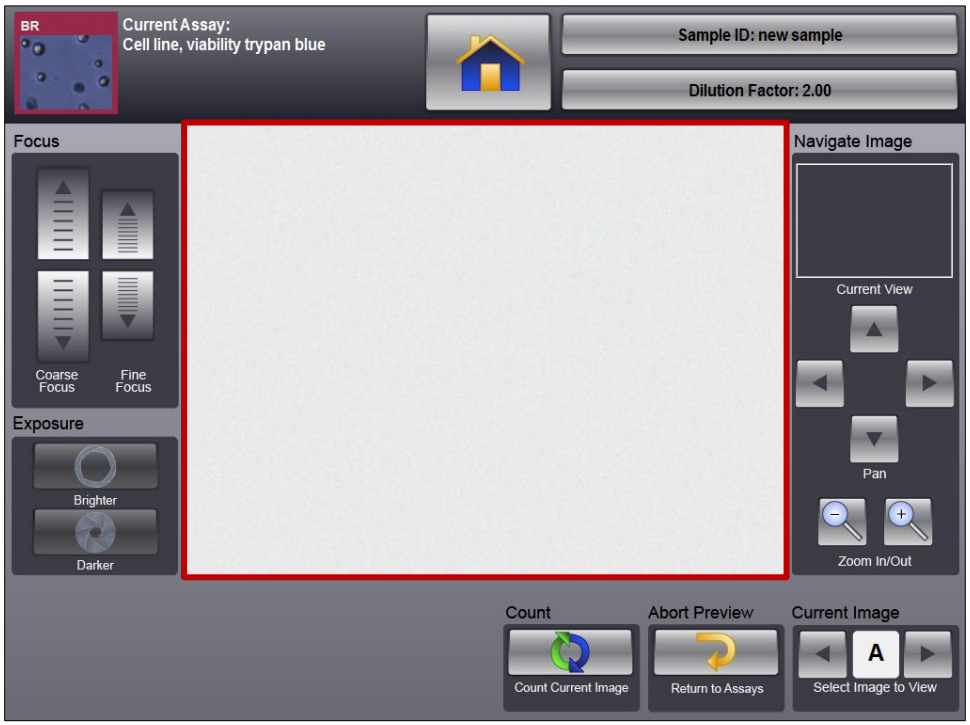


4. The system takes the background image and displays a pop-up indicating when the image has been saved. Tap **Continue**.

5. Tap the **Preview** icon located on the bottom panel of the Home screen.



6. 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

7. Tap the **Home** icon located at the top of the screen to begin counting samples.

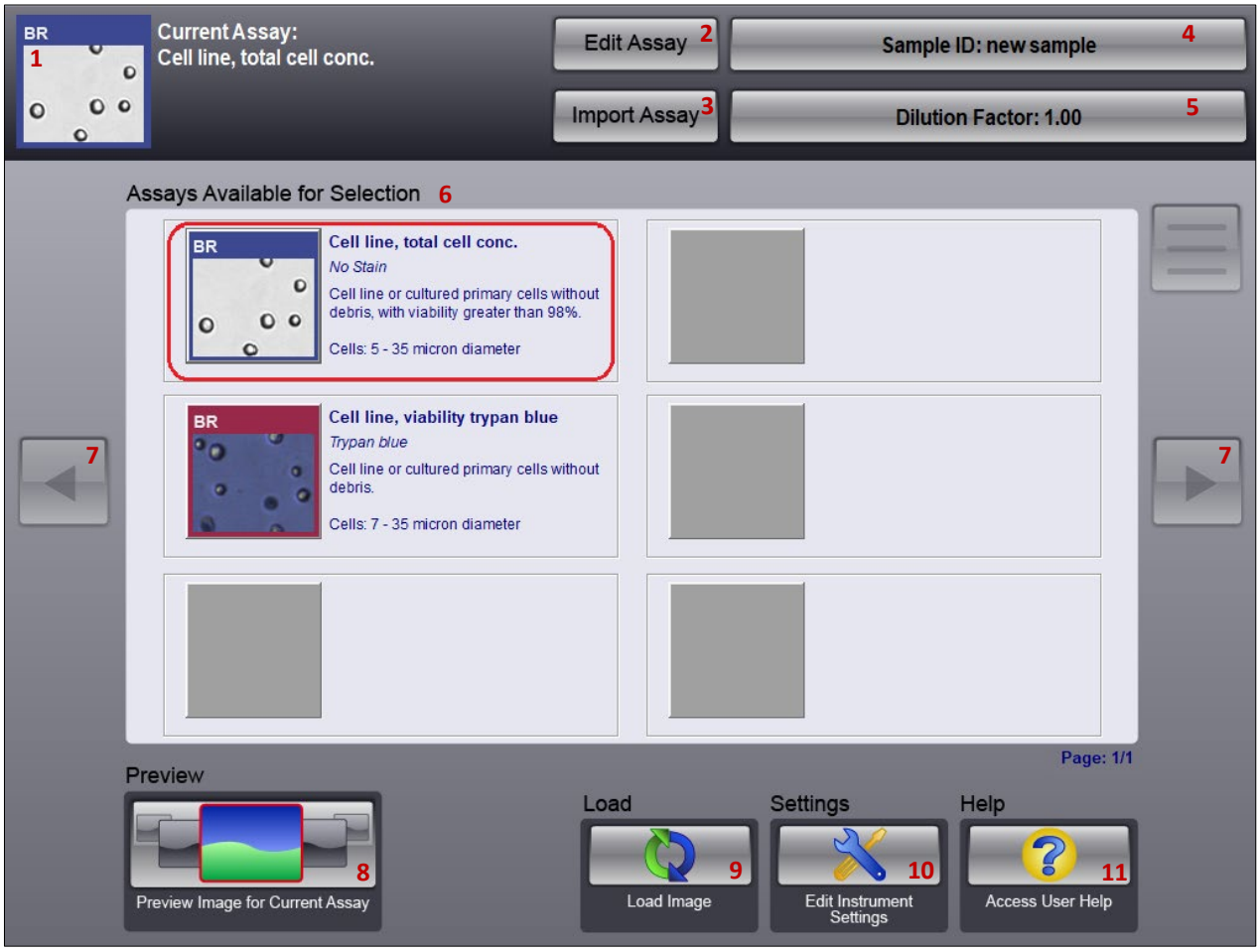
Chapter 6. Software GUI Screens

The Auto 1000 software graphical user interface (GUI) is pre-installed on the instrument and offers the fastest automated cell counting on the market. This chapter presents an overview of key screens.

Note: This instrument is a touch-screen device enabling users to interact directly with the GUI by tapping gently on screen elements (e.g., icons or buttons) using a finger or stylus. As an alternative, a USB mouse may be connected.

HOME SCREEN

The *Home Screen* (also referred to as the main screen) in the Auto 1000 software is described below.



1 **Current Assay Indicator** – Displays assay that is currently selected for analyzing the sample. *The current assay is highlighted in the Assays Available for Selection list with a red outline.*

2 **Edit Assay Button** – Enables users to customize the currently selected assay and cell type parameters.

3 **Import Assay Button** – Enables users to import and export assays from/to an external library.

4 **Sample ID Input Field** – Allows entry of a unique sample identifier.

5 **Dilution Factor Input Field** – Allows entry of final dilution factor for calculating accurate concentration.

6 **Assays Available for Selection List** – Displays all assays available for analyzing samples. *The current assay is highlighted in this list with a red outline.*

7 **Left/Right Arrows** – If the *Assays Available for Selection List* extends beyond a single screen, enables users to scroll left or right to view all assays.

8 **Preview Icon** – Displays a preview image for the currently selected assay.

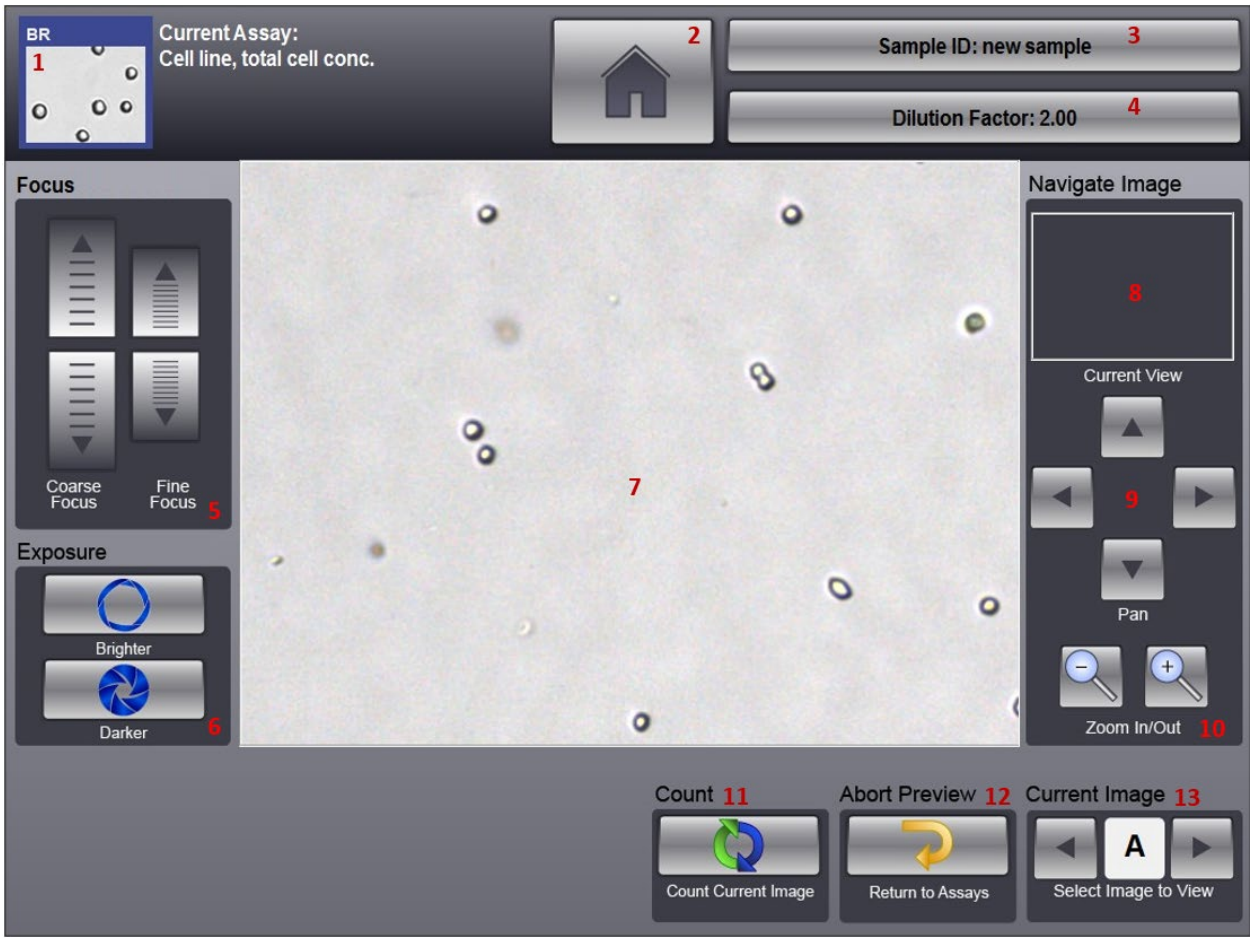
9 **Load Icon** – Loads previously saved sample images for analysis.

10 **Settings Icon** – Enables users to edit instrument and graphical user interface (GUI) settings.

11 **Help Icon** – Provides access to online Help contents and resources, instructions for submitting a support ticket, and Nexcelom contact information.

PREVIEW SCREEN

Tapping the **Preview** icon from the Home screen allows users to preview images for the current assay.



1 **Current Assay Indicator** – Displays assay that is currently selected for analyzing the sample.

2 **Home Icon** – Returns to Home screen for assay selection.

3 **Sample ID Input Field** – Allows entry of a unique sample identifier.

4 **Dilution Factor Input Field** – Allows entry of a final dilution factor for calculating accurate concentration.

5 **Focus Control** – Refines fine and coarse focus adjustment of the current sample preview. *Not enabled if a saved image has been loaded for display.*

6 **Exposure Control** – Adjusts the exposure time of the current sample preview. *Not enabled if a saved image has been loaded for display.*

7 **Viewing Pane** – Displays area of sample image as shown in the *Current View* for the *Current Image selected*. Use the *Current Image Control* to switch between available images.

8 **Navigate Image Control, Current View** – Displays a red rectangle indicating area of sample image being displayed. *As you move the Pan and Zoom In/Out controls, the Current View will change accordingly.*

9 **Navigate Image Control, Pan** – Moves the area of sample image being displayed (indicated by the red rectangle in *Current View*) in the direction of arrows selected.

10 **Navigate Image Control, Zoom In/Out** – Zooms in or out of sample image being displayed (indicated by the red rectangle in *Current View*).

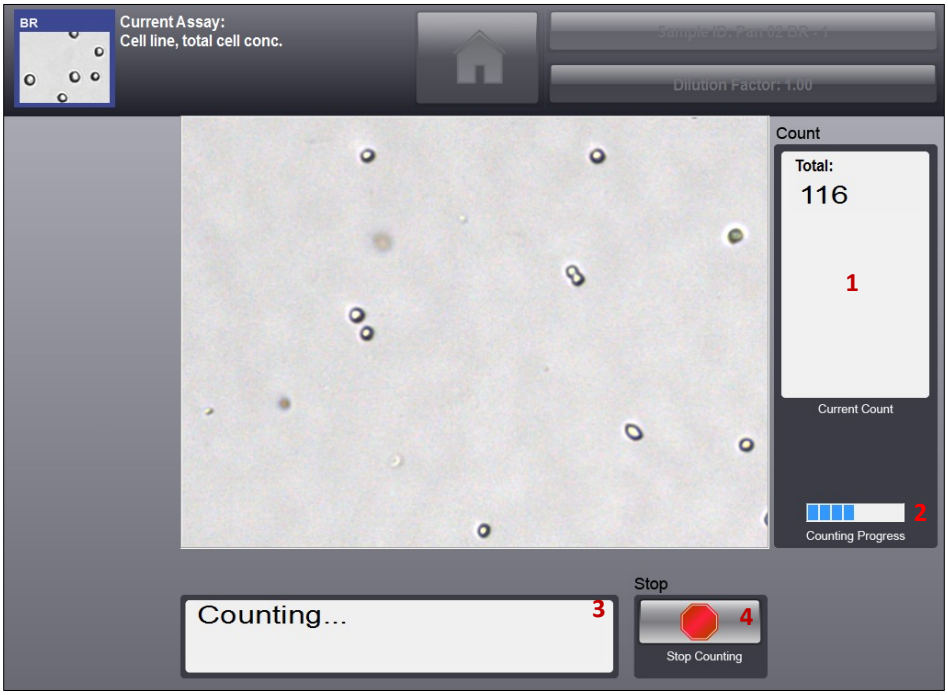
11 **Count Icon** – Initiates counting of the sample.

12 **Abort Preview Icon** – Cancels the current sample preview and returns to the Home screen.

13 **Current Image Control** – Switches between images *A, B, C* and *D* of the sample. *Multiple images were taken during the counting process representing counting chamber fields of view.*

Counting Screen

Tapping the **Count** icon from the Preview screen initiates counting of the sample. Counts displayed in the *Count Total* area will vary based on the current assay (e.g., *Total* for assays containing live cells with no stain; *Total, Live and Dead* for assays stained with trypan blue).



1 **Count Total** – Displays cell count as it accumulates throughout the counting process.

2 **Counting Progress Bar** – Indicates completion level of the counting process.

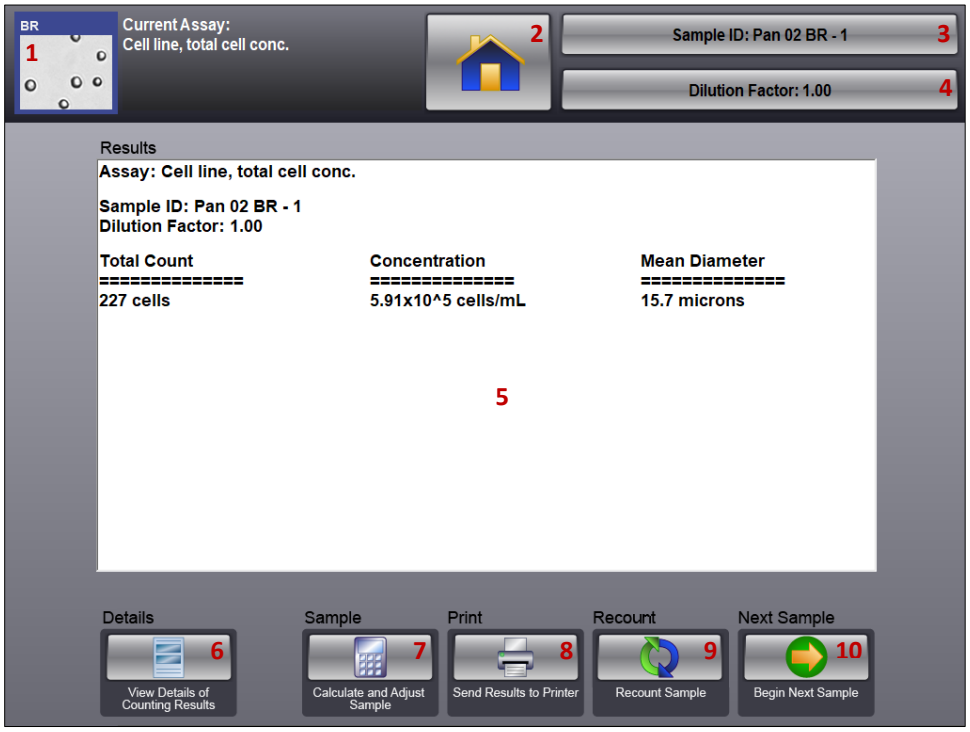
3 **Status Indicator** – Displays *Counting…* status indicating that count has been initiated.

4 **Stop** – Enables users to abort the counting process and return to the Home screen.

When the counting process is complete, the *Count Results* screen will automatically be displayed.

Count Results Screen

Count results displayed will vary based on the current assay (e.g., *Total Count*, *Concentration* and *Mean Diameter* for assays containing live cells with no stain; *Total/Live/Dead Count*, *Total/Live/Dead Concentration*, *Total/Live/Dead Mean Diameter* and *Viability* for assays stained with trypan blue). The format determining how these results are presented is defined by the *Result Template* selected for the assay which can be customized to meet the needs of your organization. See *Managing Count Result Templates* on page *52* for details.



1 **Current Assay Indicator** – Displays assay that is currently selected for analyzing the sample.

2 **Home Icon** – Returns to the Home screen.

3 **Sample ID Input Field** – Allows entry of a unique sample identifier.

4 **Dilution Factor Input Field** – Allows entry of a final dilution factor for calculating accurate concentration. *If dilution factor is modified after count is complete, results will be updated automatically*.

5 **Results Pane** – Displays cell count results for the sample. *The format used for presenting results is defined by the assay Result Template. See Managing Count Result Templates on page 52 for details.*

6 **Details Icon** – Displays Count Details screen enabling users to view counted cells, save count data and images, and generate histogram based on cell size.

7 **Sample Icon** – Launches *Sample Adjustment* tool to calculate the sample adjustment needed to obtain desired concentration or target cell number. *See Calculate Sample Adjustment on page 33 for details.*

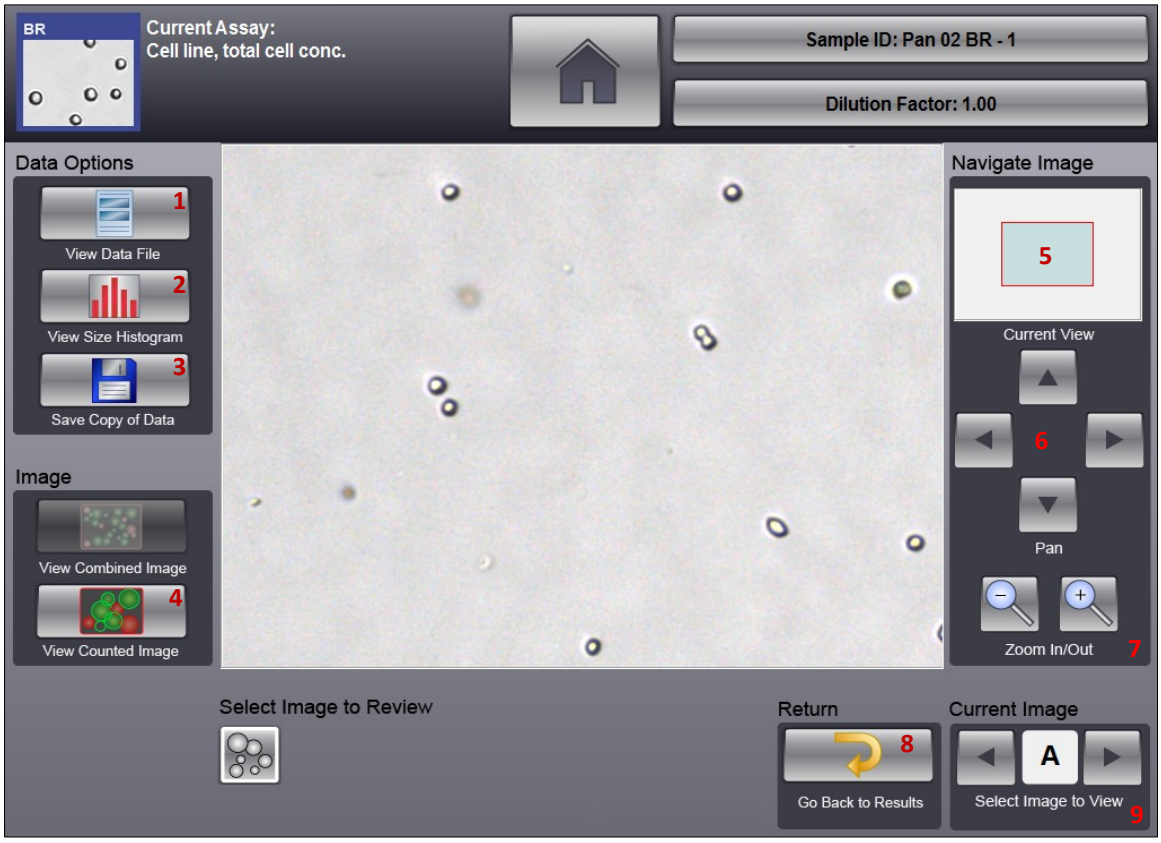
8 **Print Icon** – Sends the count results to a printer. *The format used for presenting results is defined by the assay Print Template. See Managing Count Result Templates on page 52 for details.*

9 **Recount Icon** – Re-analyzes images for current scan and performs a new count. *Parameter settings may be updated between recounts*.

10 **Next Sample Icon** – Select to start a preview of a new sample using the same Assay settings

Count Details Screen

Tap the **Details** icon from the Count Results screen to view count result details. *Data Options* are available to analyze and save results, or you can *View Counted Image* displaying a graphic overlay that outlines counted cells.



1 **View Data File Icon** – Displays data file of count results. Use the scroll bar at the bottom of the screen to view all columns and/or the **Print** icon to send the file to the printer. *Tap the **Save Copy of Data** icon to save current count results before viewing the data file if the Auto Save feature has not been enabled.*

2 **View Size Histogram Icon** – Launches *Cell Size Analysis* tool to display cell size data for your sample using a histogram plot. *See View Cell Size Analysis on page 32 for details.*

3 **Save Copy of Data Icon** – Select to save the current count results and images.

4 **View Counted Image** – Shows cell image with green/red outlines indicating live/dead cells counted.

5 **Navigate Image Control, Current View** – Displays a red rectangle indicating area of sample image being displayed. *As you move the Pan and Zoom In/Out controls, the Current View will change accordingly.*

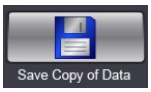
6 **Navigate Image Control, Pan** – Moves the area of sample image being displayed (indicated by the red rectangle in *Current View*) in the direction of arrows selected.

7 **Navigate Image Control, Zoom In/Out** – Zooms in or out of sample image being displayed (indicated by the red rectangle in *Current View*).

8 **Return Icon** – Returns to the Count Results screen.

9 **Current Image Control** – Switches between images *A, B, C* and *D* of the sample. *Multiple images were taken during the counting process representing counting chamber fields of view.*

SAVING COUNT RESULTS



To save sample count results, tap the **Save Copy of Data** icon on the Count Details screen.

In the Save Options window, indicate the file types to be saved (e.g., text file, raw image .png file, .jpg image file and/or counted .jpg image file). *Only raw images can be loaded into the Auto 1000 for reanalysis or sent to Nexcelom Support when requesting assistance with optimization of assay/cell type parameters.*

To save count result data to a text file, tap the **Save Results to Text File** option to display as *On*. Tap **Save** and respond to the confirmation prompt indicating the saved file location by tapping **Save** again.

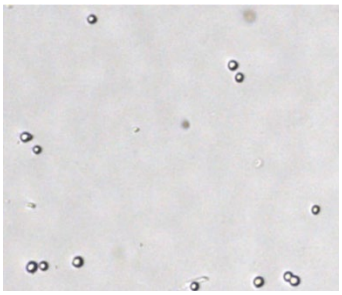
Note: Count results are saved by default to a single .txt file in which rows (logged with a date/time stamp and Sample ID) are appended each time data is saved. You can set the default folder location and file name for this file stored in instrument settings. See *Defining Default Data File/Image Folder Locations* on page *60* for details.

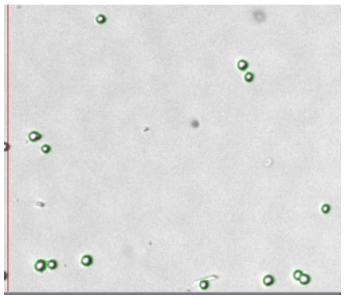
To save image files, tap one or more file type options to display as *On*. Tap **Save** and then navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**. Click **Continue** in response to confirmation prompt.

VIEWING COUNTED CELLS

To view a graphic overlay that highlights counted cells by circling them with color-coded outlines, tap the **View Counted Image** icon on the Count Details screen.

Once this option is selected, a green outline appears around cells being counted. This can help you identify if a cluster is being counted as one cell or two cells (as shown in the images below).





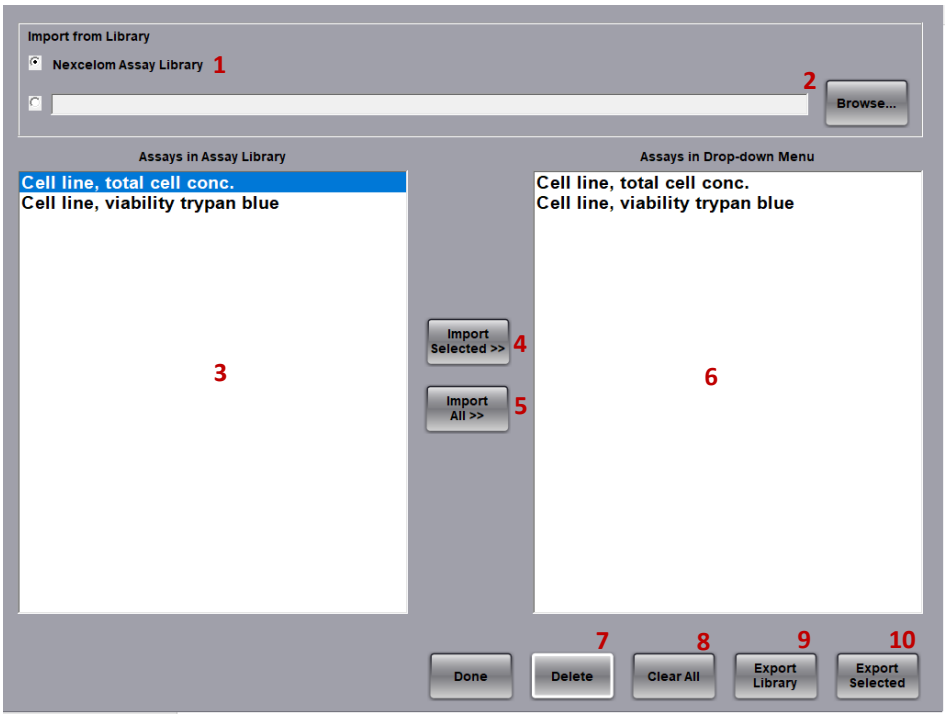
Note: When viewing counted cells, a red line may appear along edges of the Viewing Pane identifying the outer perimeter of the counting area. *Positioning of this line will change as you navigate around edges of Current View pane using Pan and Zoom In/Out controls.* Any cells appearing on or outside of this red line are *not* counted.

If testing for cell viability, selecting this option displays a graphic overlay that shows a green outline around counted live cells and a red outline around counted dead cells.

IMPORT/EXPORT ASSAY SCREEN

Assays define image analysis parameters used in sample detection. Users can import assays from the built-in *Nexcelom Assay Library* installed with the Auto 1000 software or import custom assays from an external location.

Once a library is selected, you can highlight assays from the library (displayed in the pane on the left) and import them to appear in the *Assays Available for Selection* list available on the Home screen (displayed in the pane on the right). Assays selected to appear in the list can also be exported to create a new library.



1. Selects **Nexcelom Assay Library** to display Nexcelom assays in the *Assays in Assay Library* pane.
2. Selects a custom library to display available assays in the *Assays in Assay Library* pane. If no library name is displayed, tap the **Browse** button and navigate to an external assay library.
3. List of assays in the currently selected Assay Library.
4. Imports highlighted cell types (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. List of assays to be displayed in the *Assays Available for Selection* list on the Home screen.
7. Deletes any highlighted assay in the drop-down menu (displayed in the right pane).
8. Clears all assays in the drop-down menu (displayed in the right pane).
9. Exports all drop-down menu options (displayed in the right pane) to create an Assay Library.
10. Exports selected drop-down menu options (displayed in the right pane) to create an Assay Library

Importing Assays

Used to import assays from the Nexcelom Assay Library or from external custom libraries. Individual assays can then be selected to appear in the *Assays Available for Selection* list displayed on the Home screen.

FROM NEXCELOM ASSAY LIBRARY

The **Nexcelom Assay Library** is available by default and if selected in the Import/Export Assay screen, available assays are displayed in the *Assays in Assay Library* pane.

1. In the *Import from Library* section of the Import/Export Assays screen, ensure that the **Nexcelom Assay Library** radio button is selected.
2. In the *Assays in Assay Library* pane (displayed on the left), highlight an assay to be imported.
3. Tap **Import Highlighted >>** to import highlighted assay to appear in the *Assays in Drop-down Menu* pane (displayed on the right).

   Note: If all cell types are to be imported, select **Import All >>** followed by tapping **Copy All** in response to the confirmation prompt. Importing all assays is *not* recommended if the size of the library will make the *Assays Available for Selection* list in the Home screen difficult to use.

FROM CUSTOM LIBRARIES

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

   Note: If all assays are to be imported, select **Import All >>** followed by tapping **Copy All** in response to the confirmation prompt. Importing all assays is *not* recommended if the size of the library will make the *Assays Available for Selection* list in the Home screen difficult to use.

Deleting and Clearing Assays

DELETING AN ASSAY

1. Tap the assay in the *Assays in Drop-Down Menu pane* (displayed on the right) to be deleted.
2. Tap the **Delete** button.
3. In response to the confirmation prompt, tap **Delete** to confirm the deletion.
4. Confirm that assay has been removed from the *Assays in Drop-Down Menu* pane.

Note: If assay deleted was from the Nexcelom Assay Library, it can be re-imported if necessary. If assay was from a custom library or created using the Auto 1000, it may be permanently deleted unless assay was exported and saved prior to deletion.

CLEARING ALL ASSAYS

1. Confirm that all *Assays in Drop-Down Menu pane* (displayed on the right) are to be deleted.
2. Tap the **Clear All** button.
3. In response to the confirmation prompt, tap **Clear All** to confirm the deletion. Only the *Initial Assay Type* will remain as a default.

   Note: If assays cleared were from the Nexcelom Assay Library, they can be re-imported if necessary. If assays were from a custom library or created using the Auto 1000, they may be permanently deleted unless assays were exported and saved prior to deletion.

Exporting Assays

EXPORTING ASSAY LIBRARY

1. Confirm that all assays in the *Assays in Drop-Down Menu pane* (displayed on the right) are to be exported.
2. Tap the **Export Library** button, select an **Available Drive** (displayed in bottom left corner of the screen) and then navigate to a network location by tapping folder icons.
3. Choose an existing file or tap **New File** to create a file. *If necessary, create a new folder before creating a file.*
4. Use the virtual keyboard to enter a file name and tap **Done**.
5. If prompted whether to *Lock the assays from future editing*, tap **Lock** or **Unlock**.
6. Tap **Continue** to return to the Import/Export Assay screen.

EXPORTING SELECTED ASSAY

1. Tap the assay in the *Assays in Drop-Down Menu pane* (displayed on the right) to be exported.
2. Tap the **Export Selected** button, select an **Available Drive** (displayed in bottom left corner of the screen) and then navigate to a network location by tapping folder icons.
3. Choose an existing file or tap **New File** to create a file. *If necessary, create a new folder before creating a file.*
4. Use the virtual keyboard to enter a new file name and tap **Done**.
5. Tap **Continue** in response to confirmation prompt indicating the name of the file to be saved and return to the Import/Export Assay screen.

Chapter 7. Sample Preparation

This chapter describes how to prepare Cellometer counting chamber slides and load samples into the counting chambers on a slide. See *Counting Chamber Slides* on page *69* for a list of slides available for the Auto 1000.

PREPARING COUNTING CHAMBER SLIDES

1. For SD100 slides, remove protective film from both sides of the slide. *PD100 slides are peeled and ready to use.*

   Note: It may be difficult to peel protective film from slide. One method is to adhere tape to the top of film and then pull tape off (with film attached). 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 counting chamber slide on a fresh Kimwipe.
3. To prepare two samples at once, label individual counting 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 counting 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 SAMPLES INTO COUNTING CHAMBERS

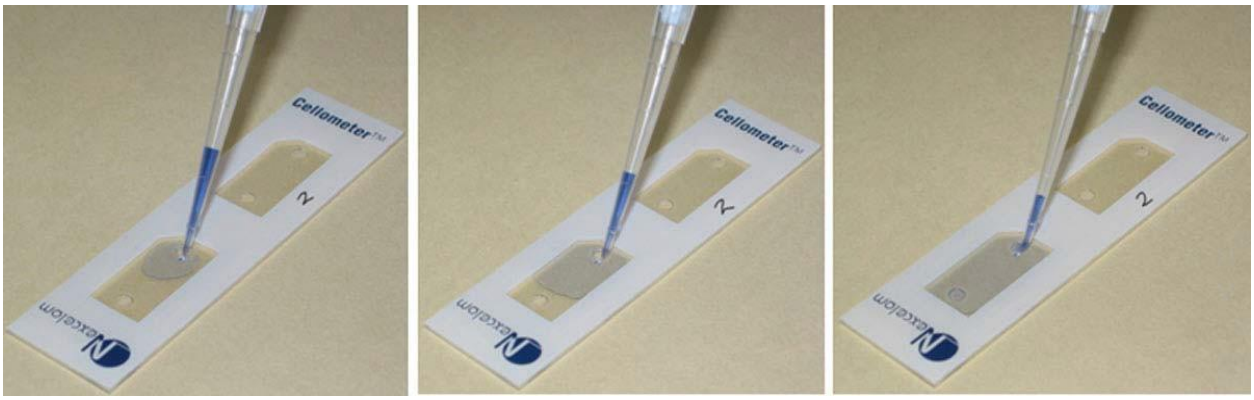
Cellometer counting chamber slides contain two independent chambers manufactured to a precisely controlled height. A cell suspension of 20 µL is pipetted into a counting chamber through its induction port.

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



Load a counting chamber by touching pipette tip to the chamber's induction port and then slowly pipetting the entire 20 µL sample all at once. Capillary force automatically spreads the sample within the chamber. Each counting chamber can hold a separate sample as there is no mixing between the two individual chambers on a slide.

Chapter 8. Counting and Analysis Workflow

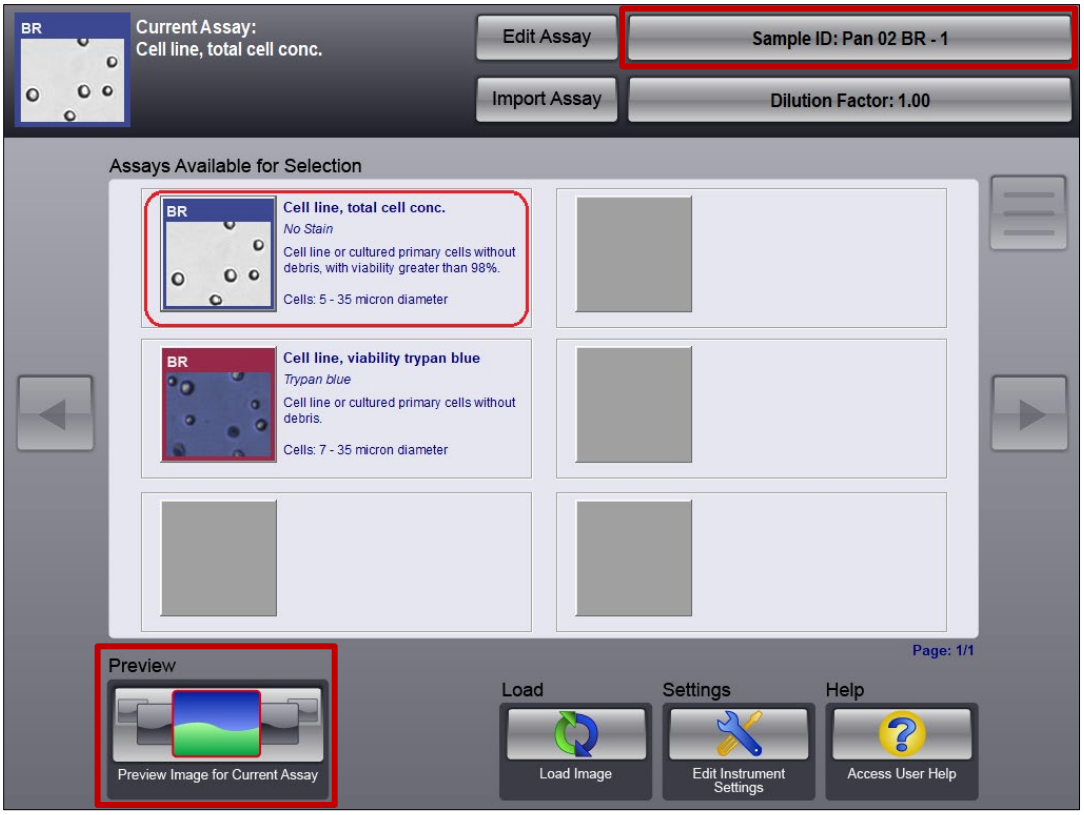
This chapter presents the basic workflow for counting cells/beads using the Auto 1000, including best practices and tips. Sample images are provided as a reference for many common cell types/beads.

PERFORM CELL COUNTING

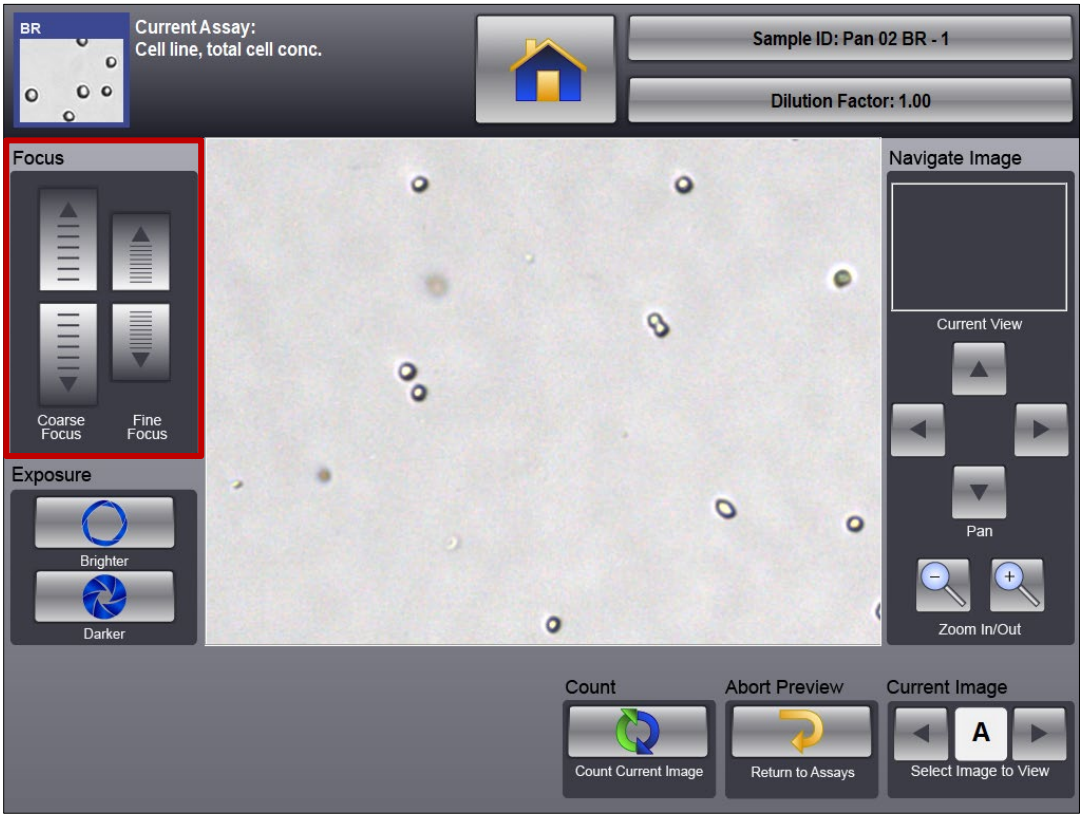
1. Power ON the instrument.
2. Slowly Pipette 20 µL of sample into counting chamber. See *Preparing Counting Chamber Slides* on page *25*.
3. Load prepared slide into Auto 1000 by inserting the counting chamber containing the sample into the Auto Sample Slot until touching the internal stop. *The chamber inside the instrument will be counted.*

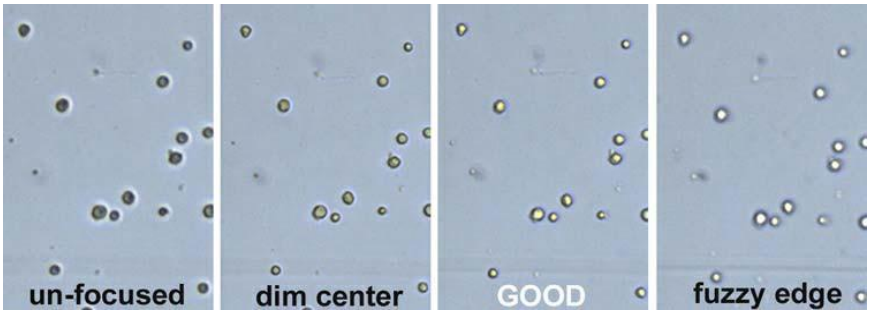
   Note: If testing for cell viability, you must stain the sample before loading it into the slide chamber.
4. In the *Assays Available for Selection* area of the Home screen, tap gently on an assay to select it. *If the assay list spans beyond the screen, arrows will be enabled on both sides to scroll back and forth through the list.*
5. Tap on the **Sample ID** input field and use the virtual keyboard to enter a sample name. Tap **Save**.
6. Tap the **Preview** icon to view images of the sample.



7. Use the **Focus** controls (*Coarse Focus* or *Fine Focus*) by tapping gently on individual controls to adjust the focus of cells in the viewing pane until proper focus is achieved. Good focus is key for obtaining accurate cell counts.

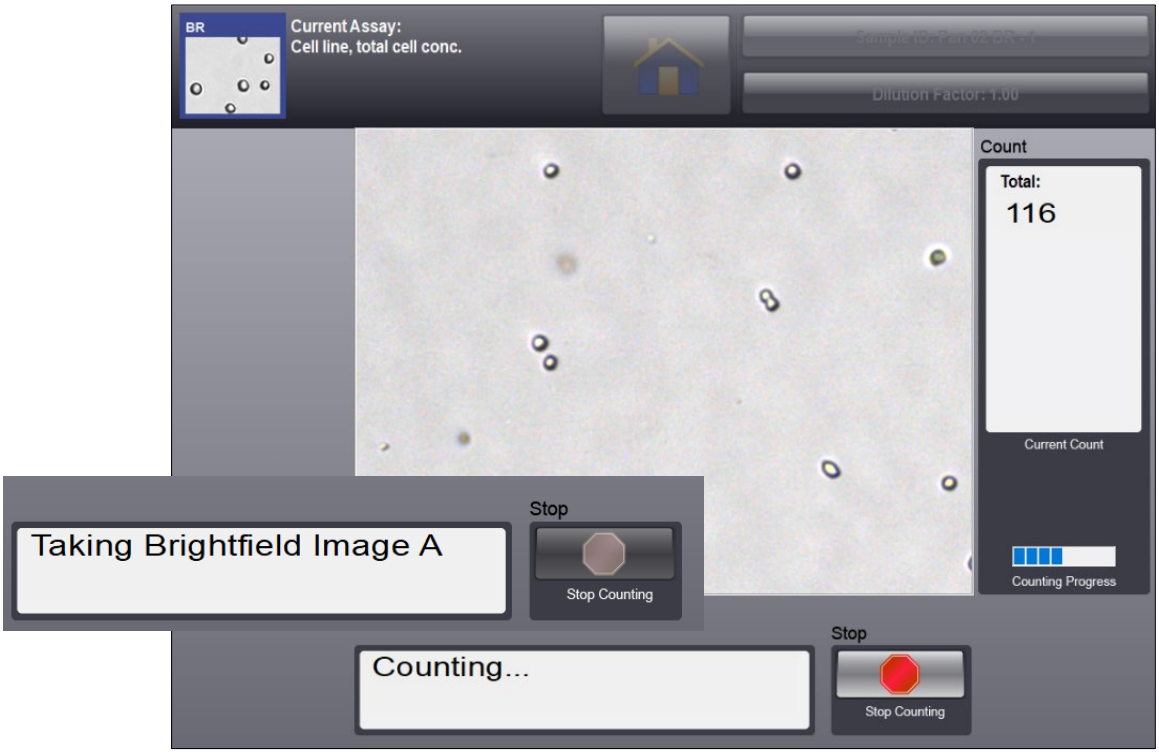


Live cells will have a bright center and a dark, crisp clearly defined edge as shown in the examples below.



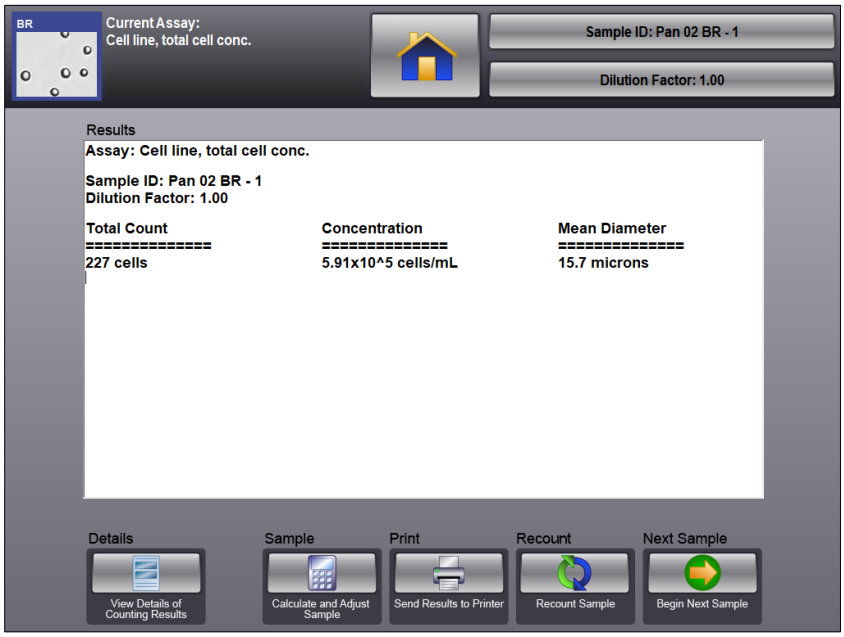
8. If necessary, tap on the **Dilution Factor** input field and change the value to indicate the final dilution factor for the sample. Tap **Save**.

9. Tap the **Count** icon while the image is displayed. A progress bar at the bottom of the software panel indicates counting in progress. A numeric counter also displays updated cell counts.



REVIEW COUNT RESULTS

1. Review the Count Results screen to confirm cells are being counted correctly. If the count results displayed appear to be correct, tap the **Details** icon to observe sample images or the **Next Sample** icon if you would like to count another sample (by repeating *Perform Cell Counting* steps on page *27*).



The format used for presenting count results is defined by the assay Result Template. See Managing Count Result Templates on page 52 for details.

2. After selecting **Details** to observe cell images, tap on the **View Counted Image** icon to view counted cells.
3. Tap the **Return** icon to return to the Count Results screen.

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 represent the entire counting chamber and include *Cell Count*, *Mean Diameter (micron)* and *Cell Concentration (cells/mL)*.
- If a viability assay 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 associated with an assay. See *Editing Cell Types* on page *41* for details.

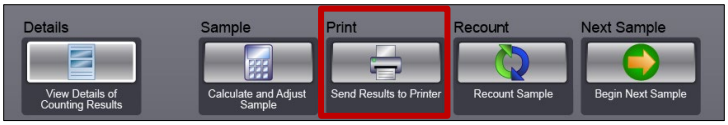
  Note: If you need help optimizing cell type parameter settings, contact Nexcelom Support for assistance at +1 (978) 327-5340 or email: support@nexcelom.com
- After modifying cell type parameters, tap the **Recount** icon to re-analyze images for current scan and perform a new count.
- Data analysis features 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 *32* and *Calculate Sample Adjustment* on page *33* for details.

Saving Data and Images

- To save count result data and images while viewing individual samples, tap the **Save Copy of Data** icon located on the Count Details screen. See *Saving Count Results* on page *21* for details. *All images captured for the sample during counting (i.e., A, B, C, D) will be saved as specified.*
- To save count result data and images automatically for all samples, enable the *Auto Save* instrument setting. See *Setting Up Auto Save Feature* on page *61* for details. *All images collected for the sample during counting (i.e., A, B, C, D) will be saved as specified.*

Printing Data

- Once a count has been performed, results can be printed by selecting the **Print** icon from the bottom panel of the Count Results screen. *The format used for presenting count results is defined by the assay Print Template. See Managing Count Result Templates on page 52 for details.*



- To print count results automatically for all samples, enable the *Auto Print* instrument setting. See *Setting Up Auto Print Feature* on page *62* for details.

VIEW CELL SIZE ANALYSIS

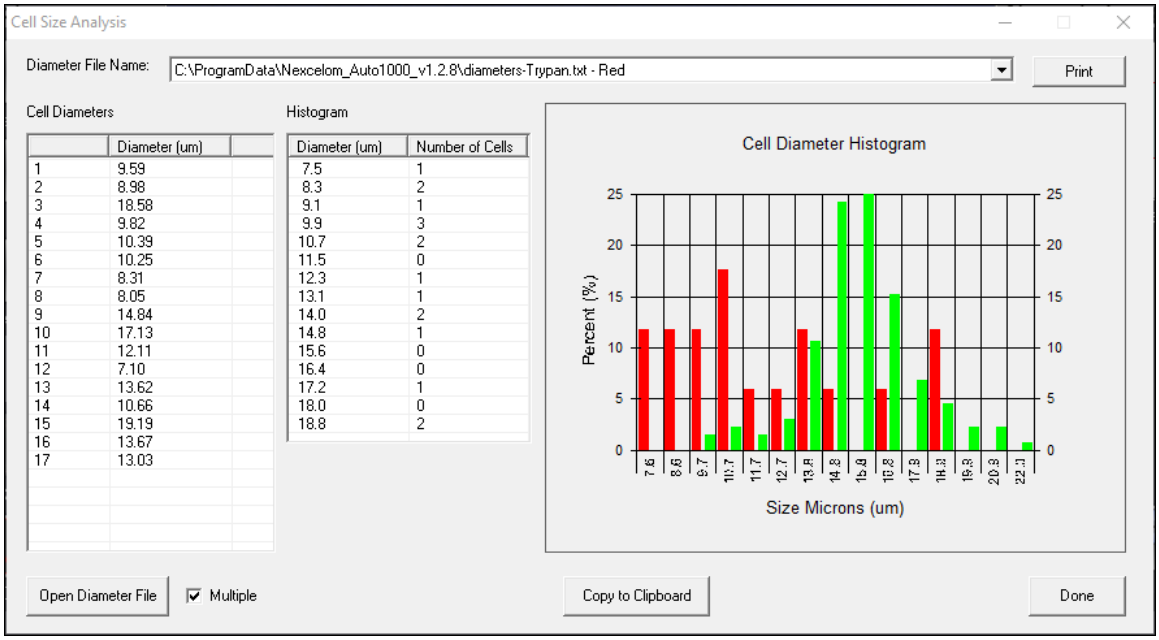
The *Cell Size Analysis* tool displays cell size data from count results by generating a histogram that enables users to optimize cell diameter parameters for the sample (e.g., to exclude debris or very large cells) and refine count data. By overlaying data from multiple samples into a single histogram, users can perform 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). Tap the **View Size Histogram** icon on the Count Details screen 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.

1. If more than one color is used for bars in the graph, choose available .txt files in the *Diameter File Name* field to toggle the data displayed in the *Cell Diameters* and *Histogram* areas, reflecting specific values for each set of colored bars. *The number of colored bars displayed will vary based on the current assay.*
2. To overlay data from multiple samples into a single histogram, tap the **Open Diameter File** button and navigate to additional diameter files.
3. Tap the **Copy to Clipboard** button to copy the graph to your Windows clipboard so it can be inserted into another application or the **Print** button to send it to a printer.

The histogram below shows cell size distribution for a trypan blue assay and contains two sets of colored bars – *Red* and *Green* (i.e., colors are assigned to bars similar to how the graphic overlay highlights counted cells). Data displayed in the *Cell Diameter* and *Histogram* areas reflects only dead cell count results (as the "- Red" .txt file is currently selected in the *Diameter File Name* field). *A "-Green".txt file containing only live cell count results is also available for selection in the Diameter File Name drop-down.*

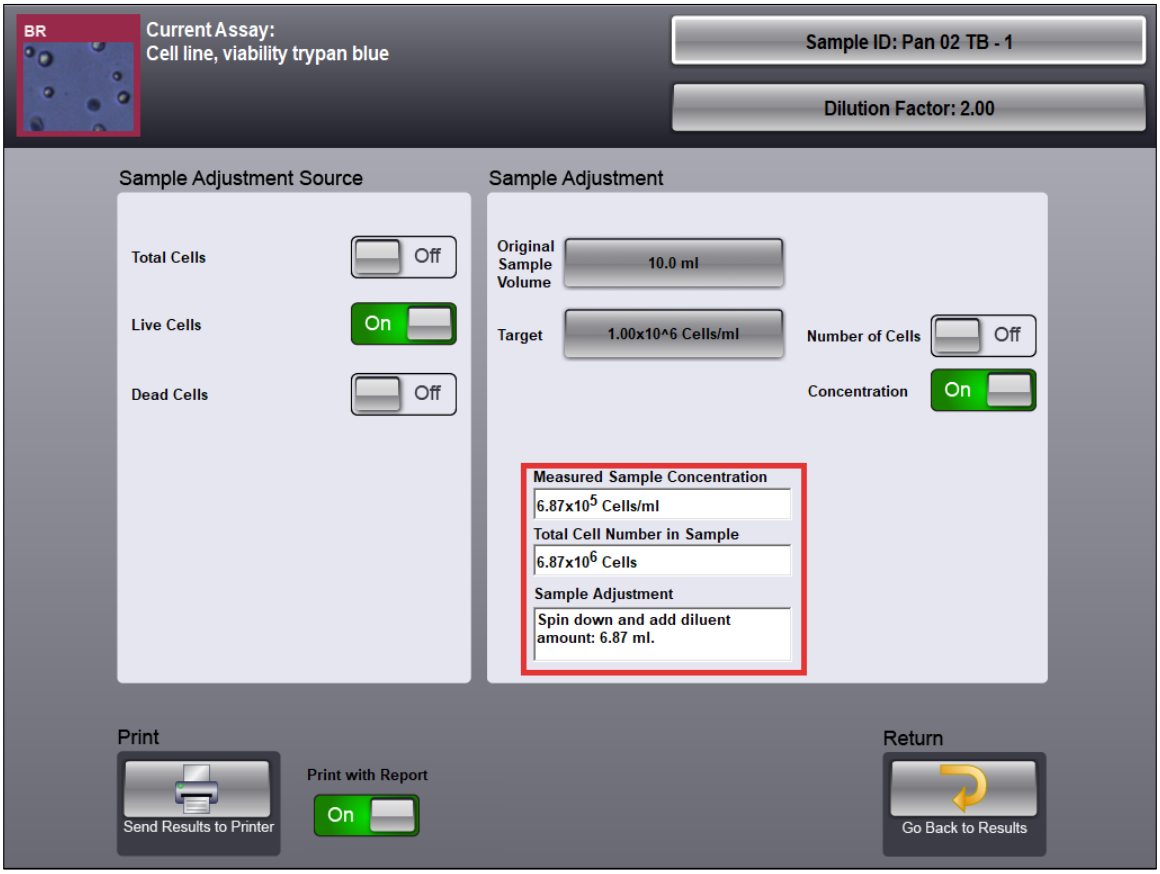


CALCULATE SAMPLE ADJUSTMENT

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. Tap on the **Sample** icon located at the bottom of the Count Results screen.
2. Indicate *Sample Adjustment Source* by choosing from available options. *Sources displayed will vary based on the current assay*.
3. Enter **Original Sample Volume** by tapping the input field to edit displayed value.
4. Indicate whether **Target** concentration value to be entered will refer to a *Number of Cells* or *Concentration*.
5. Enter **Target** concentration by tapping the input field to edit displayed value.



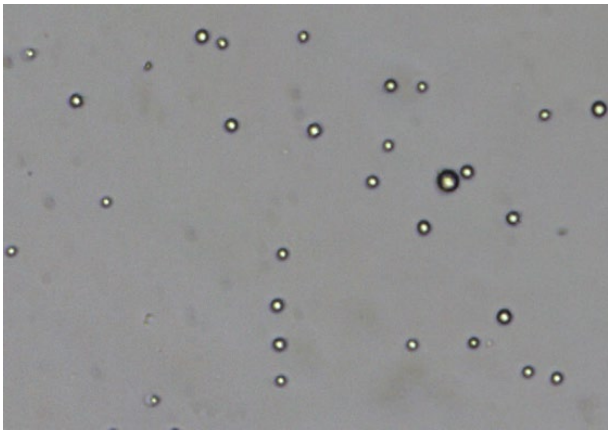
6. View calculation results for *Measured Sample* Concentration and *Total Cell Number in Sample*.
7. Perform *Sample Adjustment* instructions to achieve the cell sample goal.

REFERENCE OF CELL TYPE/BEAD SAMPLE IMAGES

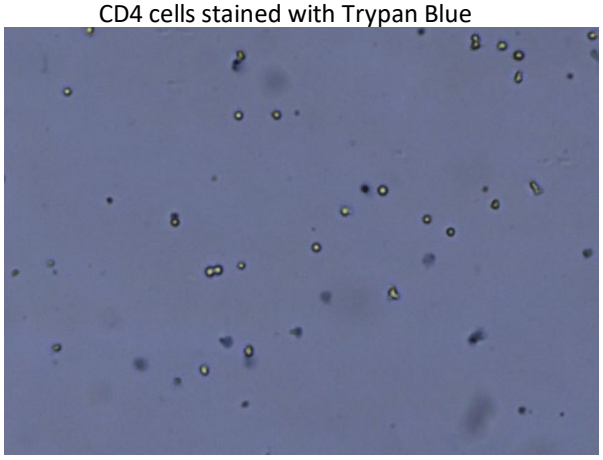
Common cell type and bead sample images taken by the Auto 1000 are presented for your reference below.

Cell Type Sample Images

Jurkat Cells

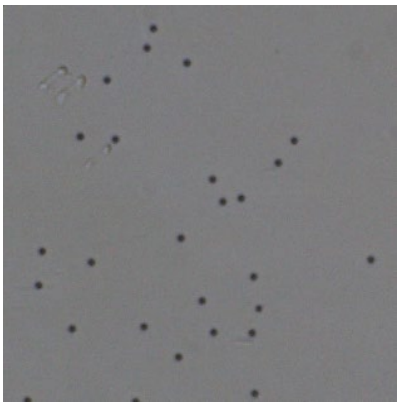


CD4 cells stained with Trypan Blue

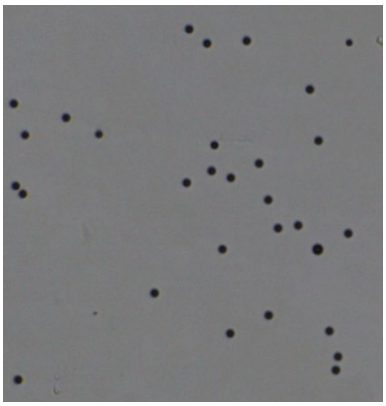


Bead Sample Images

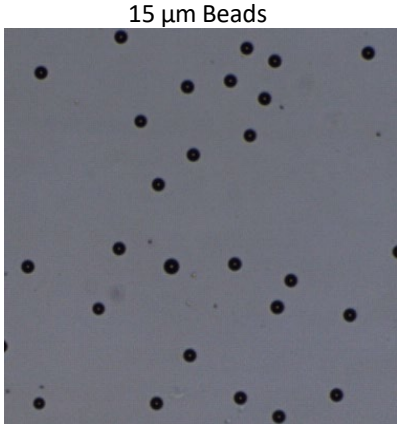
5 µm Beads



10 µm Beads



15 µm Beads



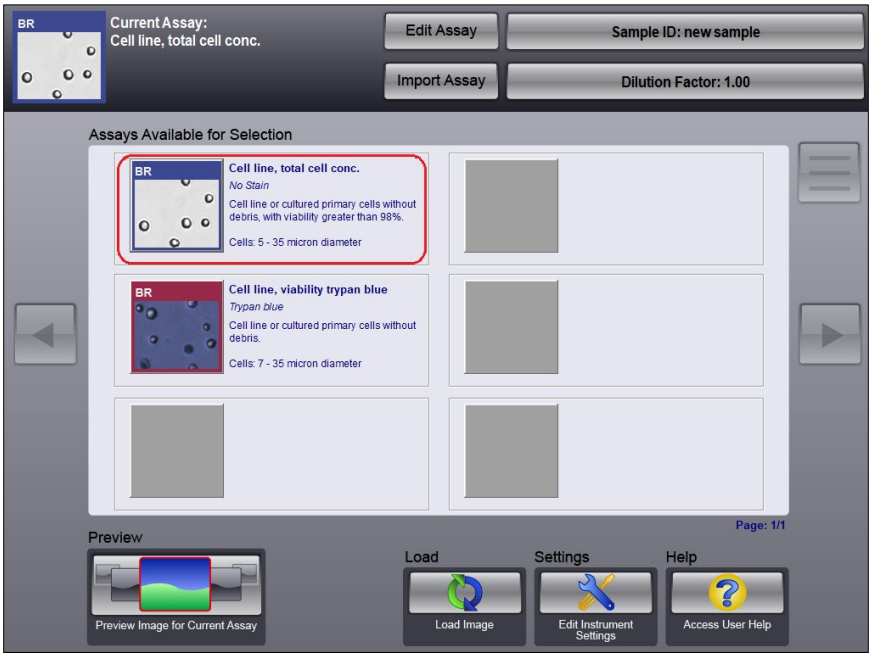
Chapter 9. Assays

This chapter describes customizable assays that can be modified to assist with cell counting and analysis.

The currently selected assay is highlighted in the *Assays Available for Selection* list with a red outline and is also displayed as the *Current Assay* in the top left corner of the screen.

DEFAULT ASSAYS

The Auto 1000 software provides two assays that are displayed by default in the *Assays Available for Selection* list displayed on the Home screen: *Cell line, total cell conc.* and *Cell line, viability trypan blue*

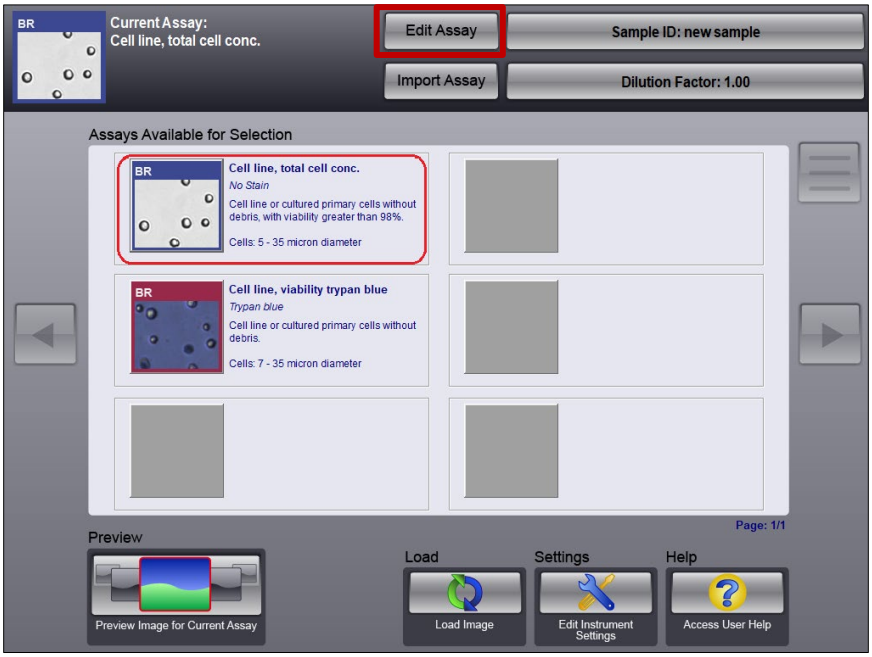


Users can edit parameter setting for assays displayed in this list and save them with a new name, or import additional assays as necessary from either the built-in *Nexcelom Assay Library* or an external network location. See *Importing Assays* on page *23* for details.

EDITING ASSAY PARAMETERS

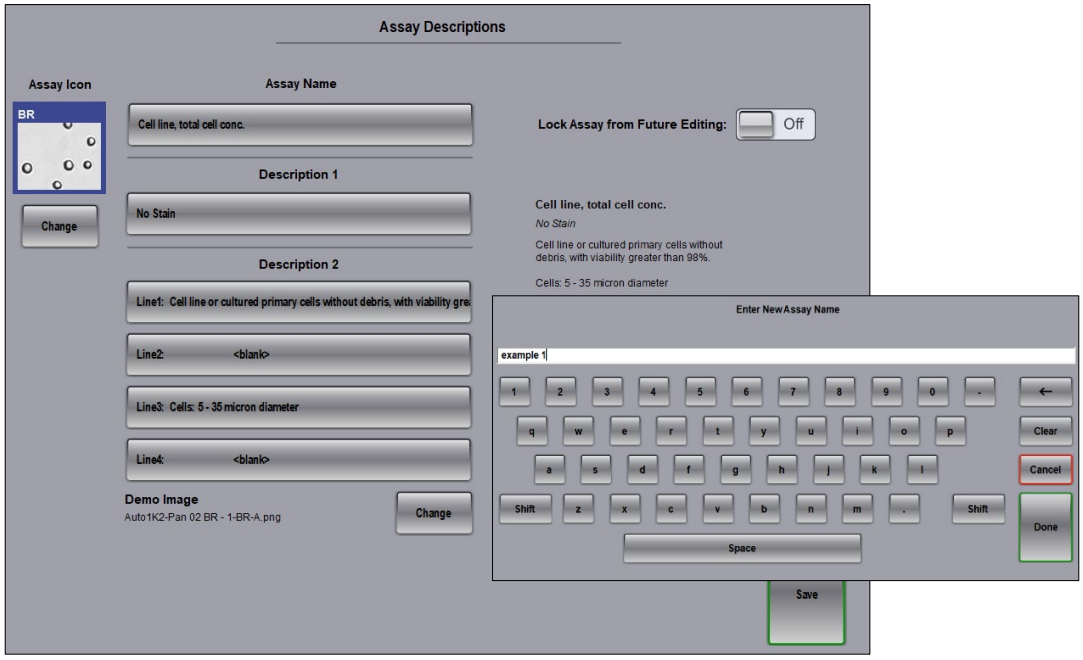
Follow these steps to modify parameters for an assay or to create a new assay:

1. On the Home screen, select an assay in the *Assays Available for Selection* list and tap **Edit Assay.**



2. In the top section of the Assay Parameters screen, tap **Edit.**

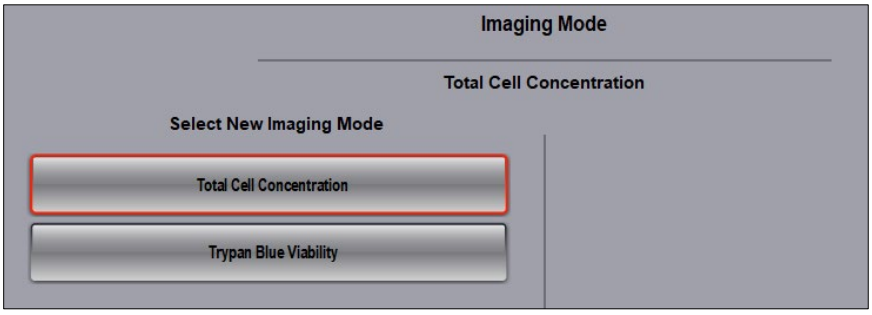
3. On the Assay Descriptions screen, enter a new **Assay Name** using the virtual keyboard that appears when you tap on the field. Tap **Done** when you are finished to close the keyboard.



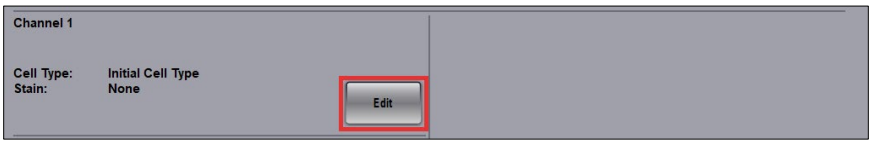
Note: Upon closing, you will be prompted to *Change the cell type name to match the new assay name or keep the old cell type name*. The cell type name is displayed in the *Channel 1* area on the Assay Parameters screen. See *Editing Cell Types* on page *41* for details about editing cell types.

4. Modify details for **Description 1** and/or **Description 2** (*Lines 1-4*) as appropriate for the assay.
5. Tap **Save** to save the assay description and return to the Assay Parameters screen.
6. To select an imaging mode for the assay that is different from what is currently displayed (e.g., *Brightfield Only* in the screen below), tap **Edit** in the *Imaging Mode* area.

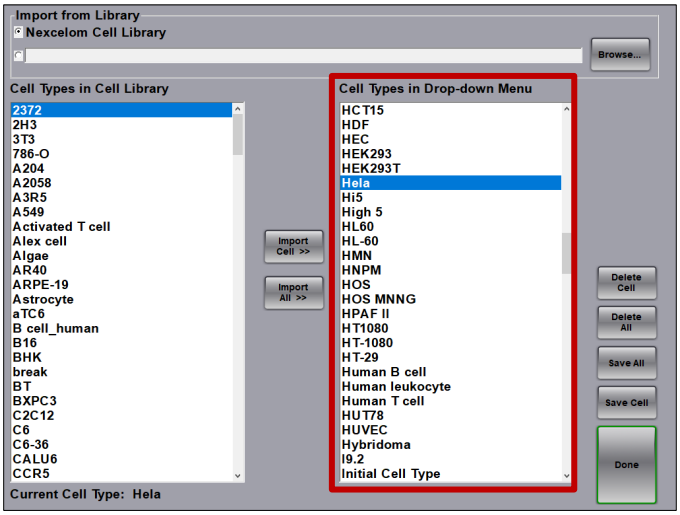
7. Select an imaging mode for the sample – *Total Cell Concentration* (for cells with no stain) or *Trypan Blue Viability* – and tap **Save**. Respond to confirmation prompts as necessary.



8. To select a cell type for your assay that is different from what is currently displayed (e.g., *Initial Cell Type* in the screen below), select **Edit** in the *Channel 1* area.



9. In the Channel 1 Settings screen, tap **Select New** to display the Cell Types screen containing available cell types in the *Cell Types in Drop-down Menu* pane (displayed on the right).

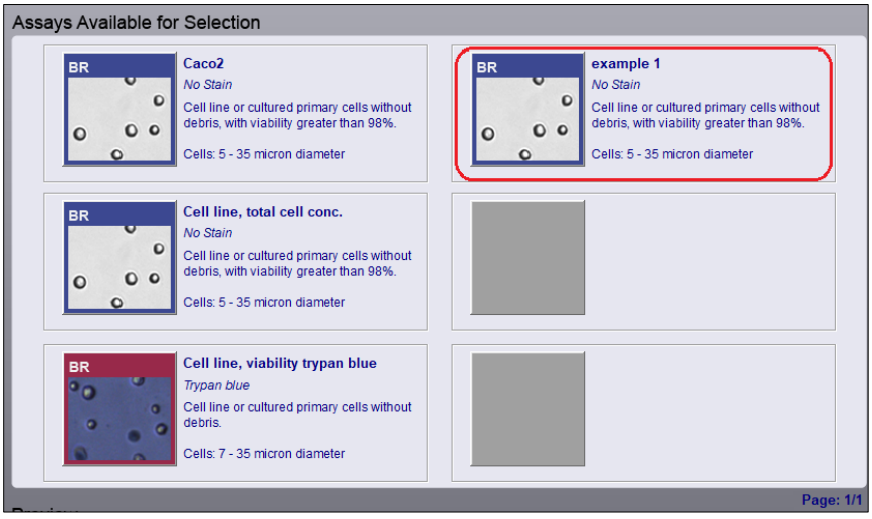


10. Select a cell type from the *Cell Types in Drop-down Menu* pane and tap **Done**.

    Note: If the cell type needed is not displayed in the *Cell Types in Drop-down Menu* pane, you can either import it from the Nexcelom Cell Library by highlighting it in the *Cell Types in Cell Library Pane* (displayed on the left) and then tapping **Import Cell >>** to copy it to the *Cell Types in Drop-down Menu* pane or import it from a custom library. See *Importing Cell Types* on page *49* for details.

11. The selected cell type is displayed in the Channel 1 Settings screen. Tap **Save**.

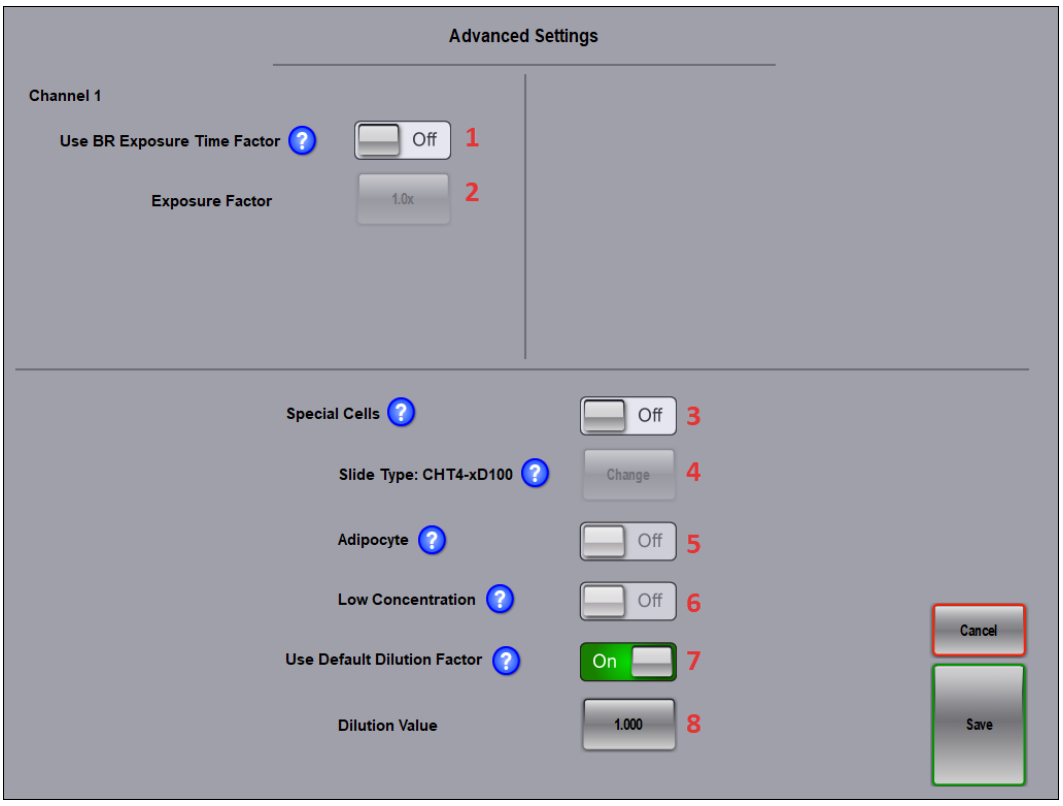
12. Tap **Save** again to return to the Home screen. The new assay appears in the *Assays Available for Selection* list.



EDITING ADVANCED ASSAY PARAMETERS

Tap the **Edit** button in the *Advanced Settings* area (bottom section) of the Assay Parameters screen to edit advanced assay parameters.

Advanced Settings can be used to vary the Brightfield exposure time factor, enable specialized counting algorithms for specific cell types, and set a default dilution factor.



1 **Use BR Exposure Time Factor** – Changes the Brightfield exposure time by the displayed factor.

2 **Exposure Factor** – Displays the factor by which to change Brightfield exposure time. ***Use BR Exposure Time Factor*** *parameter must be selected for this input field to be enabled.*

3 **Special Cells** – Applies special counting algorithms and allows for change of counting chamber slide type. *If selected, enables the* ***Slide Type***, ***Adipocyte*** *and* ***Low Concentration*** *parameters.*

4 **Slide Type** – Displays the current counting chamber slide type. ***Special Cells*** *parameter must be selected for this field to be enabled.*

5 **Adipocyte** – Applies special counting algorithms for adipocyte cells. ***Special Cells*** *parameter must be selected for this field to be enabled.*

6 **Low Concentration** – Enables counting of all four images for a sample as a single image. ***Special Cells*** *parameter must be selected for this field to be enabled.*

7 **Use Default Dilution Factor** – Enables users to set a default dilution factor for the assay.

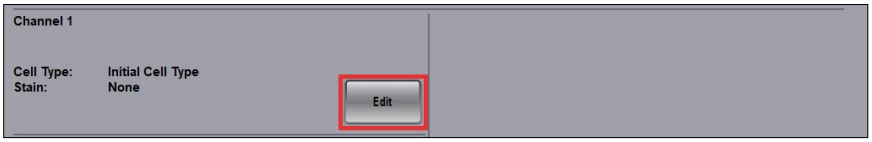
8 **Dilution Value** – Displays the dilution factor set for the assay. ***Use Default Dilution Factor*** *parameter must be selected for this input field to be enabled.*

Chapter 10. Cell Types

This chapter describes editing parameters for cell types associated with assays and lists all cell types included in the built-in Nexcelom Cell Library. In addition, it provides instructions for editing templates used for the display and printing of count results.

EDITING CELL TYPES

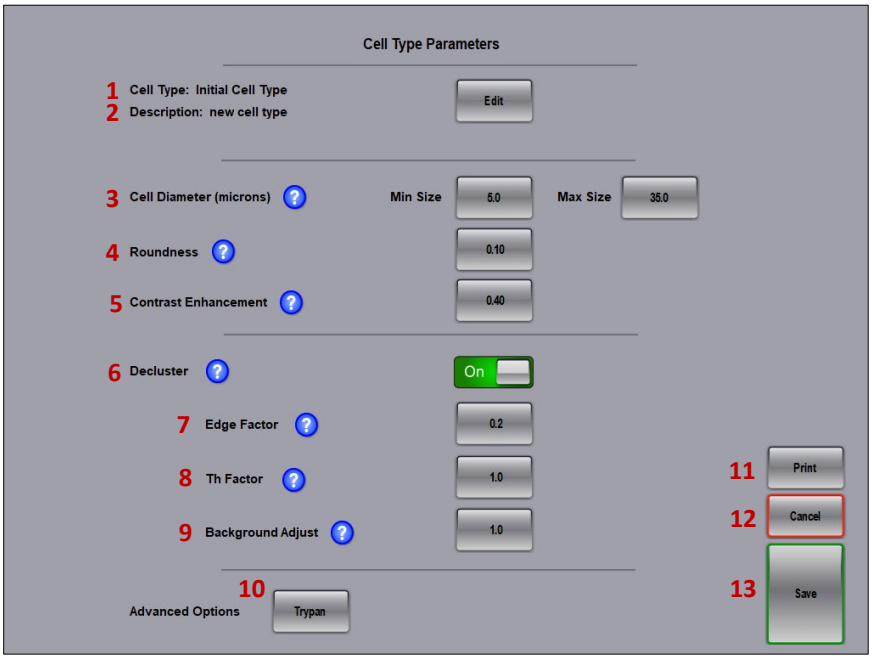
To edit the cell type for an assay, highlight the assay in the *Assays Available for Selection* list and tap the **Edit Assay** button, followed by the **Edit** button in the *Channel 1* area of the Assay Parameters screen.



In the Channel 1 Settings screen, select the **Edit** button to display the Edit Cell Type Parameters screen.

Note: To select a new cell type, tap the **Select New** button. See *Importing Cell Types* on page *48* for details.

Cell Type Parameters Screen



1 **Cell Type Name** – Identifies name given to the cell type. *Tap the **Edit** button to edit cell type name.*

2 **Description** – Contains detailed cell type description. *Tap the **Edit** button to edit cell type description.*

3 **Cell Diameter** – Indicates minimum (**Min Size**)/maximum (**Max Size**) cell diameters to be counted.

4 **Roundness** – Indicates minimum cell shape roundness factor to be counted. *Values range from 0.10 (includes all cell shapes) to 1.0 (includes only perfectly round cells)*.

5 **Contrast Enhancement** – Defines contrast enhancement value for cells in relation to the background. *Values range from 0.01 (cells with high contrast to background) to 0.90 (cells with low contrast to background); recommended value is 0.4.*

6 **Decluster** – Defines whether individual cells within a clump are to be counted. *Turning this off allows clumps to be counted as one unit if its diameter falls within the min/max diameter range.*

7 **Edge Factor** – Indicates degree to which cell edges must be enhanced for optimal declustering. *Values range from 0.0 (clearly defined edges) to 1.0 (edges difficult to distinguish from background).*

8 **Threshold Factor** – Indicates the threshold ratio between cell signal and background. *Values range from 0.0 (cell signal to background is very low) to 1.0 (cell signal to background is high).*

9 **Background Adjust** – Indicates the adjust ratio between cell signal and background. *Values range from 0.0 (cell signal to background is very low) to 1.0 (cell signal to background is high).*

10 **Advanced Options, Trypan Button** – Defines trypan blue viability cell detection parameter settings.

11 **Print Button** – Prints the cell type parameter settings.

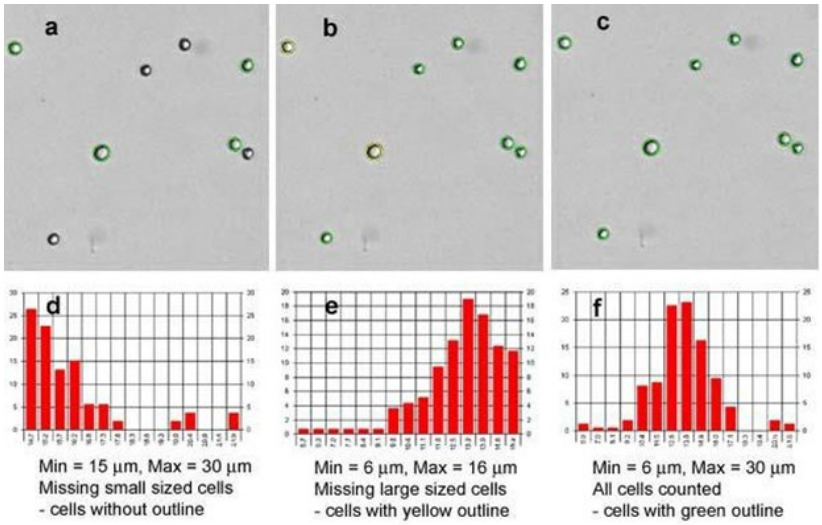
12 **Cancel Button** – Cancels changes you have made to parameter settings and returns to previous screen.

13 **Save Button** – Saves changes to parameter settings.

ADJUSTING CELL DIAMETER PARAMETERS

For each cell type, **Cell Diameter Min Size/Max Size** (in microns) parameter setting indicate the size of cells to be included in the count.

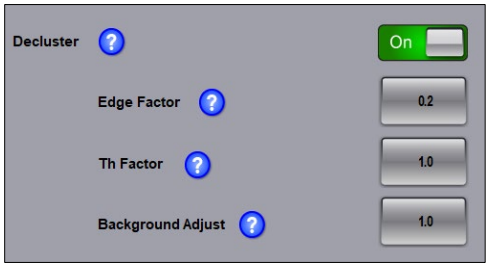
To determine if cell diameter parameters are set up properly, use the **View Counted Image** icon in the Count Details screen to display a graphical overlay outlining counted cells (e.g., green for counted cells and yellow for cells *not* counted as they are larger than the specified cell diameter).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

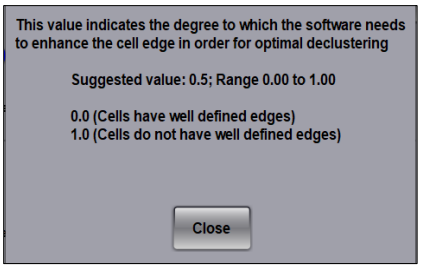


HT-29 cell count results were analyzed using different cell diameter parameter settings. Figure (a) – "Cell Diameter Min Size" size is too large. Cells smaller than 15 µm are not counted, as indicated by missing outlines. Figure (b) – "Cell Diameter Max Size" is too small. Cells larger than 16 µm are not counted, as indicated by the yellow outlines. Figure (c) – Both "Cell Diameter Min Size" and "Cell Diameter Max Size" 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 parameters.

ADJUSTING DECLUSTER PARAMETERS FOR CLUMPY CELLS

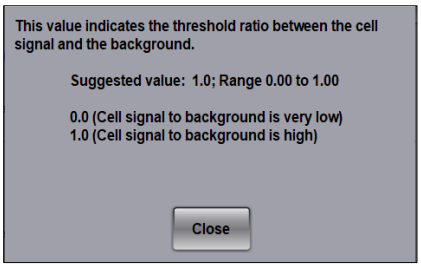
The **Decluster Edge Factor** and **Decluster Th Factor** (*Decluster Threshold Factor*) parameters may be used to separate clumpy cells (i.e., connected cells for which it is difficult to distinguish clearly defined edges). *Default values for these parameters are 0.5 and 1.0, respectively.*





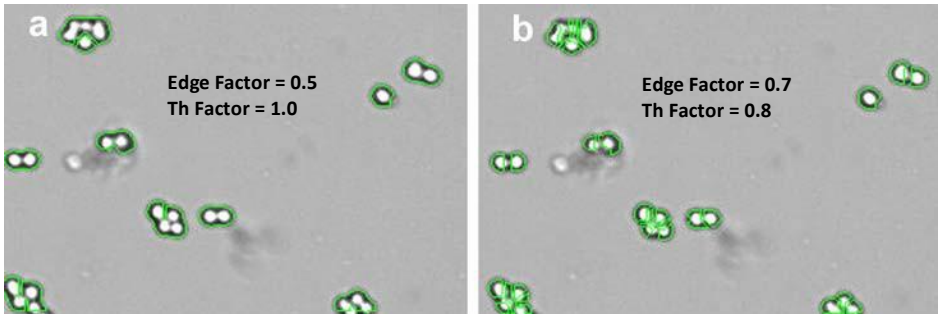
For most cell types, the default values for these parameters are adequate to separate clumpy cells. However, some cell types (such as MCF7 and PC12) contain cells having dim boundaries within the clump. In these cases, decluster parameters can be modified to visually separate clumpy cells in the captured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



SAMPLE CLUMPY MCF7 CELLS

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

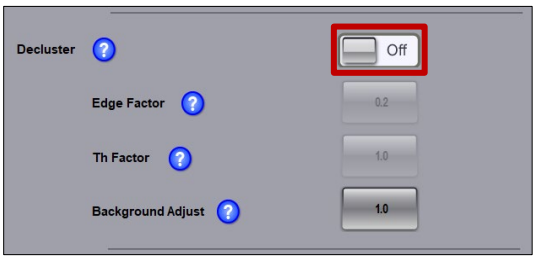


MCF7 cell count results were analyzed using different "Decluster" parameter settings. Figure (a) shows default decluster settings (cells within clumps are not fully separated), resulting in MCF7 concentration of 6.5 x 10^5 cell/mL. When modified declustering settings are used as shown in figure b, cells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

DISABLING DECLUSTER FOR SMALL CELLS

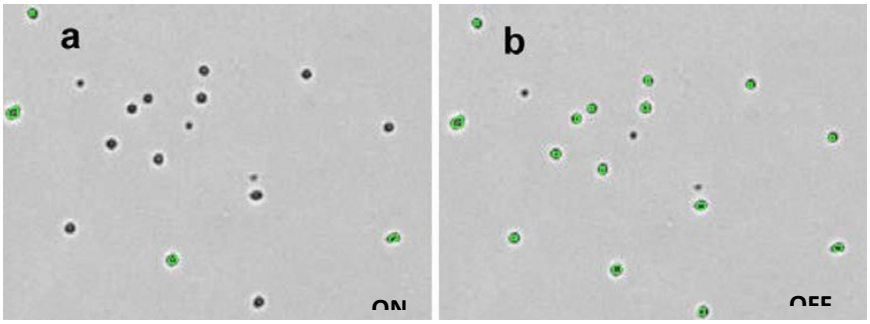
Examples of small size cells are mouse splenocytes, lymphocytes, thymocytes, and PBMCs. Their cell diameters are typically less than 7 µm.

The Auto 1000's focus position for really small cells may influence cell counting when the *Decluster* feature is enabled (i.e., the **Decluster** parameter is *not* enabled). *This feature works in conjunction with the **Contrast Enhancement** value on the Cell Type Parameters screen.*



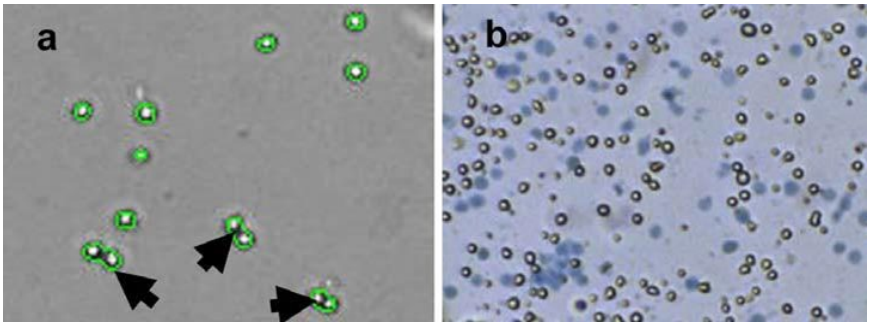
For the purpose of the following example, cell type parameter settings as shown in this screen were used to count mouse thymocyte cells in low concentration (i.e., cell counts less than 1300). Images explaining count results for various scenarios are presented.

Mouse thymocyte count results using variations in the **Decluster** parameter are shown below.



Mouse thymocyte count results are analyzed with Decluster parameter enabled in figure (a) and disabled in figure (b). The green outlines indicate counted cells. When Decluster parameter is not enabled, more cells are counted.

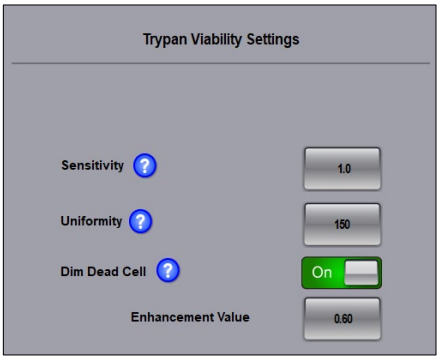
However, some samples contain clumpy cells, as shown in figure (a) below. Other samples have a very high cell concentration as shown in figure (b). For these samples, the *Decluster* feature is enabled and the **Contrast Enhancement** value was reduced (to a value of *.70*).



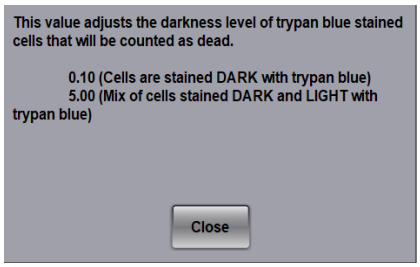
Cell type parameters for high concentration or clumpy small-sized cells.

ADJUSTING TRYPAN VIABILITY PARAMETERS

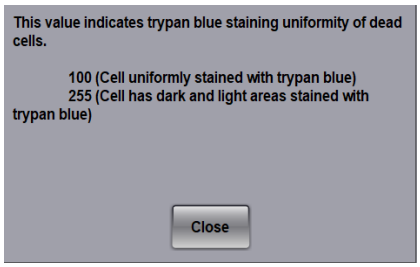
Using default settings for *Trypan* viability parameters provides the fastest counting speed for standard cell types stained a solid dark blue by trypan blue. As some cell types exhibit different morphology *after* being stained, it may be necessary to adjust parameters for accurate detection of the trypan blue population as indicated below. From the Cell Type Parameters screen, tap on the **Trypan** button in the *Advanced Options* area to display the Trypan Viability settings screen.



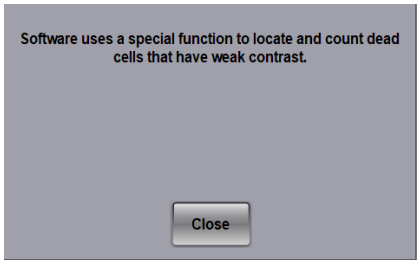
Sensitivity – Adjusts the darkness level of trypan blue stained cells to be included in dead cell count. *Values range from 0.10 (detects very dark stained cells to 5.00 (detects more mixed staining populations).*



Uniformity – Indicates trypan blue staining uniformity to be included in dead cell count. *Values range from 100 (stained cells are all uniform in color) to 255 (stained cells have non-uniform dark and light areas).*



Dim Dead Cell – When feature is enabled (ON), helps to detect very dim stained dead cells.



Enhancement Value – *Dim Dead Cell feature must be selected for this field to be enabled.* Refines the division between the background and cells with low contrast (i.e., no defined edges). *Suggested Enhancement value is 0.60; values range from 0.04 (stained cells have medium contrast to background) to 0.90 (stained cells have very low contrast to background).*

WEAK BLUE DEAD CELLS

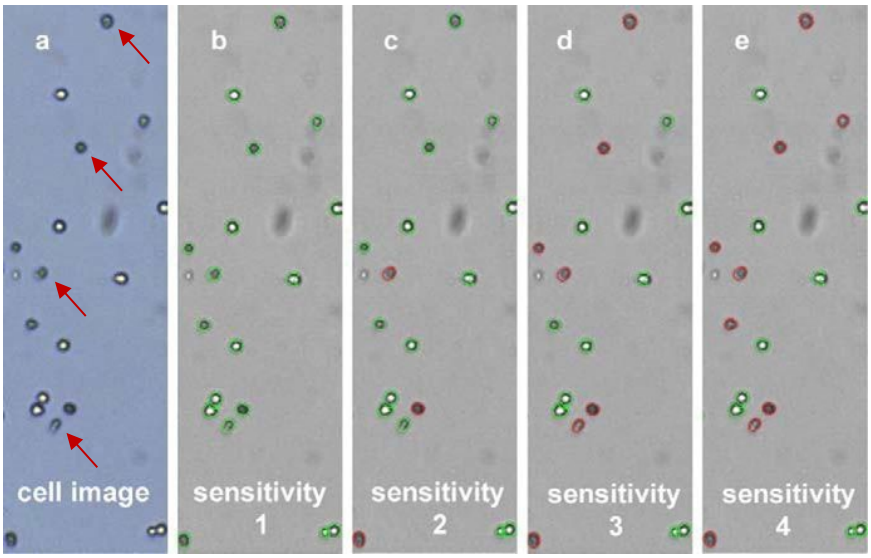
To pick up weak blue dead cells, enable the **Dim Dead Cell** feature and slowly increase the **Enhancement Value** to further refine the division between the background and cells with low contrast (i.e., no defined edges).

Note: It is a best practice to use the **Dim Dead Cell** feature in conjunction with other *Trypan* viability parameters (i.e., *Sensitivity*, *Uniformity or Enhancement Value*) to avoid overcounting.

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 in the figures below.



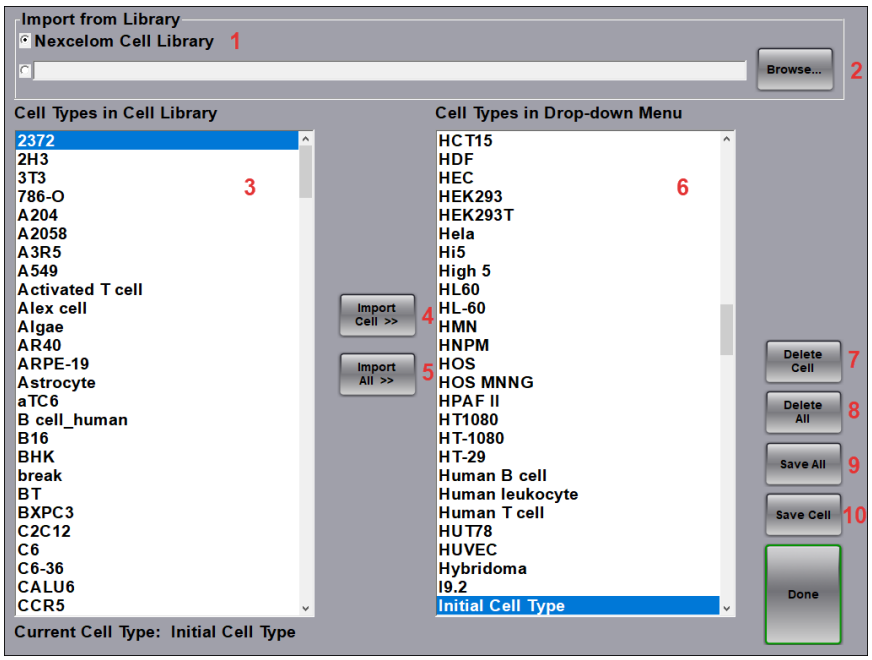
CHO cell count results were analyzed using different "Sensitivity" settings. Figure (a) shows CHO cell image while figures (b) – (e) show an incremental increase in "Sensitivity" parameter from 1 to 4, respectively. Green outlines indicate live cells while red outlines indicate dead cells. Both live/dead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

IMPORTING CELL TYPES

Cell Types define image analysis parameters used in cell sample detection. The *Import Cell Type Screen* is used to import either standard cell types from the *Nexcelom Cell Library* installed with the Auto 1000 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 in Drop-down Menu* pane (displayed in the pane on the right).

To change the cell type selected for the current assay, highlight a cell in the *Cell Types in Drop-down Menu* pane and tap the **Done** button.



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, tap 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 List of cell types to be displayed in the drop-down menu.

7 Deletes the highlighted cell type in the drop-down menu (displayed in the right pane).

8 Deletes all cell types in the drop-down menu (displayed in the right pane).

9 Saves all cell types to a library file (e.g., *MyCells*) in the specified network location.

10 Save the highlighted cell type to a library file (e.g., *MyCells*) in the specified network location.

Cell Type Drop-down Defaults

The Auto 1000 displays *all* standard cell types from the Nexcelom Cell Library in the *Cell Types in Drop-down Menu* pane of the Import Cell Type screen as initial defaults. Users can import additional custom cell types to appear in this drop-down as necessary from an external network location.

Note: If cell types contained in the Nexcelom Cell Library can no longer be found (i.e., they have been cleared by users over time), they may be imported again from either the Nexcelom Cell Library or an external library as indicated below. See the *Nexcelom Cell Library* on page *51* for a list of all available standard 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 in Drop-down Menu* pane.

FROM NEXCELOM CELL LIBRARY

The **Nexcelom Cell Library** is available by default and if selected in the Import 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 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. Tap **Import Highlighted >>** to import highlighted cell type to appear in the *Cell Types in Drop-down Menu* pane (displayed on the right).

   Note: Although the **Import All >>** button can be used to import *all* cell types, it is not recommended if the size of the cell library will make the *Cell Types in Cell Library* pane difficult to use.

FROM CUSTOM LIBRARIES

1. In the *Import from Library* section of the Import Cell Type screen, tap the custom cell library radio button to choose a custom library (if available) or tap **Browse** to navigate to folder containing a custom library and double-tap 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. Tap **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 in Drop-down Menu* pane, **Import All >>** can be used to import *all* cell types from the library followed by tapping **Copy** in response to the confirmation prompt.

Deleting Cell Types

1. To delete a single cell type from the *Cell Types in Drop-Down Menu pane* (displayed on the right), highlight the cell type and tap the **Delete Cell** button. To delete *all* cell types from the *Cell Types in Drop-Down Menu* pane, tap the **Delete All** button.
2. In response to the confirmation prompt, tap **Delete** or **Delete All** to confirm the deletion.
3. Confirm cell type has been removed from *Cell Types in Drop-Down Menu* pane.

   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 1000, it may be permanently deleted unless cell type was exported/saved prior to deletion.

Saving Cell Types to a File

1. To save a single cell type listed in the *Cell Types in Drop-down Menu* pane (displayed on the right), highlight the cell type and tap the **Save Cell** button. To save *all* cell types from the *Cell Types in Drop-Down Menu* pane, tap the **Save All** button.
2. Select an **Available Drive** (displayed in bottom left corner of the screen) and then navigate to a network location by tapping folder icons.
3. Choose an existing file or tap **New File** to create a file. *If necessary, create a new folder before creating a file.*
4. Use the virtual keyboard to enter a file name and tap **Done**.
5. If saving a single cell type, tap **Continue** in response to confirmation prompt indicating file name to be saved. If saving *all* cell types, indicate whether to *Lock the cells from future editing* by tapping **Lock** or **Unlock**, followed by **Continue** in response to confirmation prompts.
6. Tap the **Done** button to return to the Channel 1 Settings screen.

NEXCELOM CELL LIBRARY

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

2372
2H3
3T3
786-O
A204
A2058
A3R5
A549
Activated T cells
Alex cell
Algae
AR40
ARPE-19
Astrocyte
aTC6
B cell_human
B16
BHK
break
BT
BXPC3
C2C12
C6
C6-36
CALU6
CCR5
CD14
CD3
CD3_high concentration
CD4
CD8 clone
CEF
CGN
CHO-1
CHO-3
CL-1
Colo205
Cortical neurons
Cos7
CRL-1863
CT-26
CT-26-B7
CT-26-CEA
CT26-CEA-B7
D cell
D Mel
D1.1
DC145
DC-PBMC mix
Dictyoslelium
DLD
D-MEL
DU
DU145
DU145 G37
EL4
Fao
Fibroblast_human
Fibroblast_human_high density
Fibroblast_pig
FRhK4
FU5AH
G37
GHOST
GM cell
GRANTA
H157
H358
H42E
H520
H647
H9
HASMC
HBL-2
HC9
HCE4
HCT15
HDF
HEC
HEK293
HEK293T
Hela
Hi5
High 5
HL60
HL-60
HMN
HNPM
HOS
HOS MNNG
HPAF II
HT1080
HT-1080
HT-29
Human B cell
Human leukocyte
Human T cell
HUT78
HUVEC
Hybridoma
I9.2
Initial Cell Type
Initial Cell Type_Trypan
Ink-4A
INS
Jurkat
K1
K562
K9 fibroblast
KA T4
KR158
KT1
KT-1 cell
KT2
KT2 cell
KU812
L cell
L1210
L929
Large size cells
LCL
LLC-PK1
Lncap
lymph node suspension
Lymphoblast
Lymphoma
M19
Macrophage
Macrophages
MAGIC
Malme 3M
Mast
MC32A
MC32A-B7
MC-38
MC-38-B7
MCF-10A
MCF-10A4
MCF7 clumpy
MCF7 non-clumpy
MCF7_clumpy
MDA-231
MDA-MB-231
MDA-MB-435
MDA-MB-468
MDBK
MDCK-Focus
medium size cells
MEF
Melan A
Melan-3m
Melanocyte
Melanoma
Mel-STR
Mel-STV
MES-SA
MG63
MM1S
MMS
MNT1
Monocyte
MRC5
MSB1
MTA-9
MV411
N59
NCI-460
NCI-H929
ND468
Neutrophile
new cell type
NIH 3T3
NM4A
NM4A4
OCIM1
OVCAR
P19
PA-317
Pan-2
PBMC Turks
PC
PC12
PC-12
PC3
PDAC
PLHC
QNR-K22
Raji
Ramos
RAW
RCC45
RCS
RD
RD cell
ReN
RPMI7951
RPMI-7951
SaOS
SF9
SK28
SKMel28
SK-MEL-28
SKOV3
small size cells
SNU475
SW48
SW480
SW620
T cell
T cell_human
TH1
THP
Til-T
TME-7
Tn5
TS
U118
U266
U2OS
U87
U937
UACC 257
V12
V529
Vero
WIDR
Wil2S

If you would like help creating a new assay, customizing cell type parameters for an assay or creating a new cell type, send saved raw images to Support@Nexcelom.com and we will optimize a custom assay/cell type for you. See *Saving Count Results* on page *21* for details on how to save information that Nexcelom will need.

MANAGING COUNT RESULT TEMPLATES

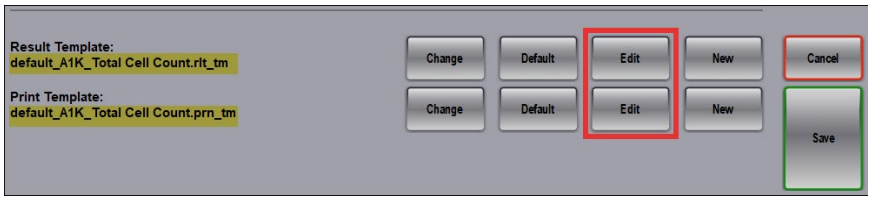
When displaying count results, templates are used to format how data is displayed on the Count Results screen and when printing data as reports. By launching the *Template Editor* available from the Imaging Mode screen, you can customize default templates and/or create a library of new templates to meet the needs of your organization.

Note: Editing templates requires use of a physical keyboard and mouse. You will be prompted to confirm these devices are connected to the instrument via USB ports before you can use the template editor.

Editing Count Result Templates

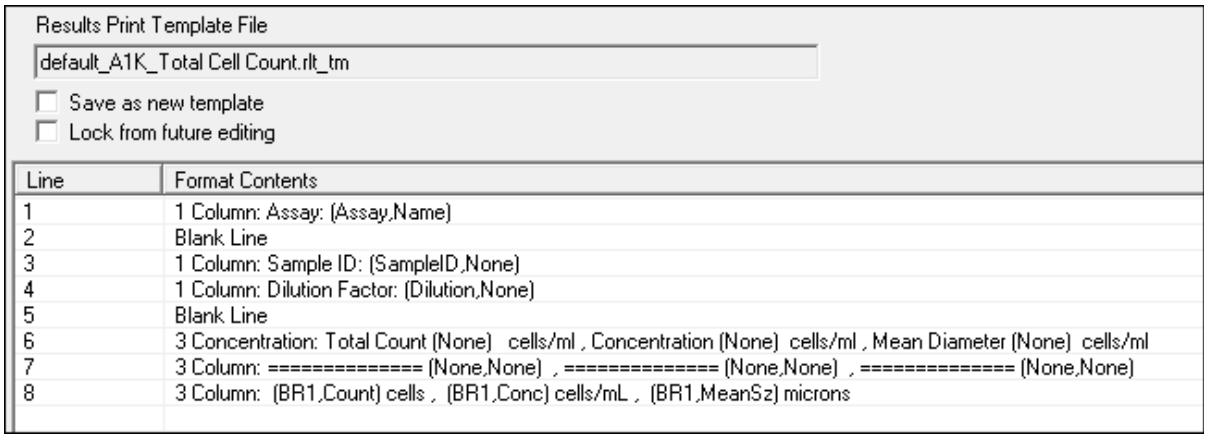
1. In the *Imaging Mode* area of the Assay Parameters screen, select the **Edit** button.

2. At the bottom of the Imaging Mode screen, tap the **Edit** button for either the *Result Template* file or the *Print Template* file to select the template you want to modify. *File extensions on result template files indicate the template type (i.e., .rlt_tm for Result Templates or .prn_tm for Print Templates).*



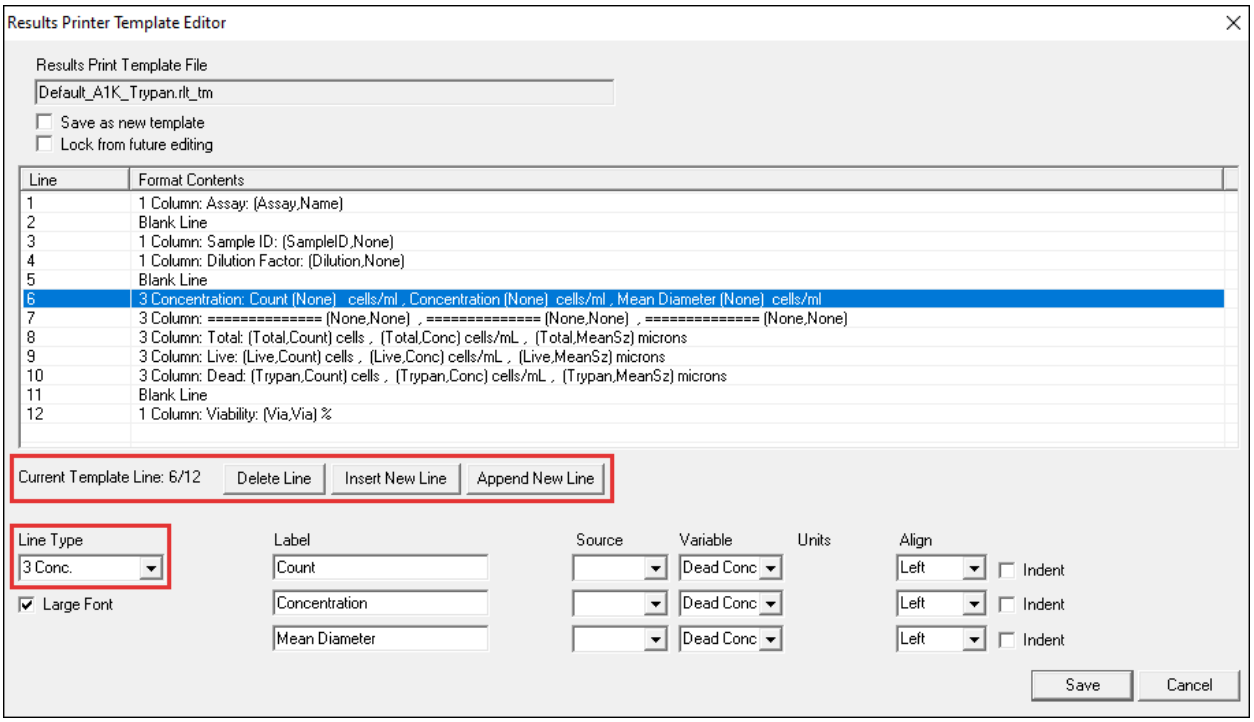
3. Connect a physical keyboard and mouse to the instrument via the USB ports and tap **Continue** in response to the confirmation prompt.

   The template editor displays the template file for the current assay, listing all *Lines* and their associated *Format Contents* that appear in the template. You can modify the template file by inserting/deleting lines, appending new lines, and changing the format content of lines as necessary.



Line	Format Contents
1	1 Column: Assay: (Assay,Name)
2	Blank Line
3	1 Column: Sample ID: (SampleID,None)
4	1 Column: Dilution Factor: (Dilution,None)
5	Blank Line
6	3 Concentration: Total Count (None) cells/ml , Concentration (None) cells/ml , Mean Diameter (None) cells/ml
7	3 Column: ========== (None,None) , ========== (None,None) , ========== (None,None)
8	3 Column: (BR1,Count) cells , (BR1,Conc) cells/mL , (BR1,MeanSz) microns

4. To modify the template by deleting, inserting or appending lines, use the mouse to position the cursor on the desired line and click the **Delete Line**, **Insert New Line** or **Append New Line** buttons. *New lines are inserted above the current line while appended lines are inserted below it.*



5. To modify the format contents of a line, use the mouse to position the cursor on the desired line and select an option from the **Line Type** drop-down (e.g., *Blank*, *Page Break*, *1-3 Columns*, *1-2 Images*, *1-3 Concentrations* and *Adjust Report*). *Fields displayed for editing format contents will vary depending on the selected line type.*

6. To edit the format content of a line, modify values in the **Label**, **Source**, **Variable**, **Units** and **Align** fields (if applicable) accordingly. Click the **Indent** check box if you want to indent the line from the left margin.

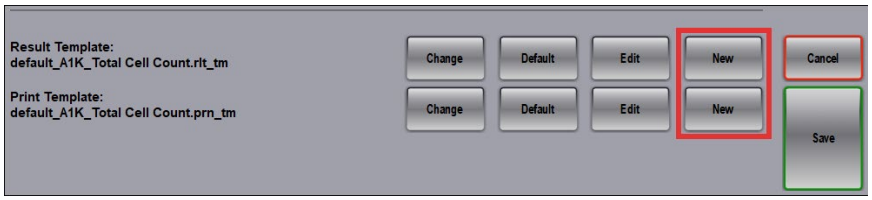
   Note: You can add up to two images per line, specifying the **Type** (*File* or *Instrument*) and alignment. *Additional fields may be displayed based on selected image type.*

7. When your changes to the template file are complete, click **Save** to return to the Imaging Mode screen.

8. Click **Save** until you return to the Home screen, select the **Preview** icon to view the image for current assay and select the **Count** icon to display count results. Confirm format of your modified *Result Template* or select the **Print** icon in the bottom panel of the screen to confirm format of your *Print Template*.

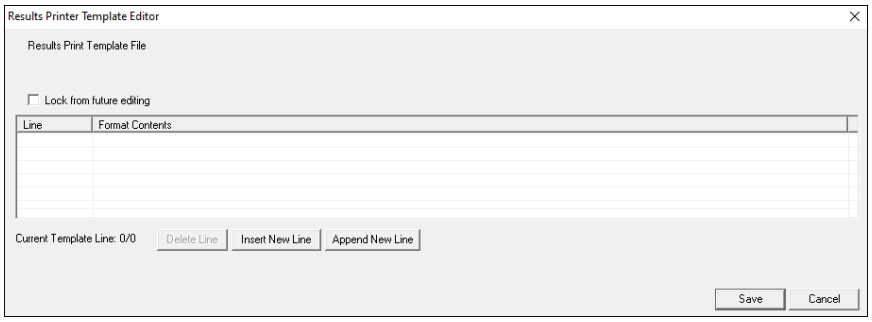
Creating New Result Templates

1. Return to the Imaging Mode screen and tap the **New** button for either the *Result Template* or *Print Template*.



2. Connect a physical keyboard and mouse to the instrument via the USB ports and tap **Continue** in response to the confirmation prompt.

3. The template editor displays a new (blank) template file. Insert or append new lines to the template and set up the format content for each line as appropriate. See *Editing Count Result Templates* on page *52* for details.

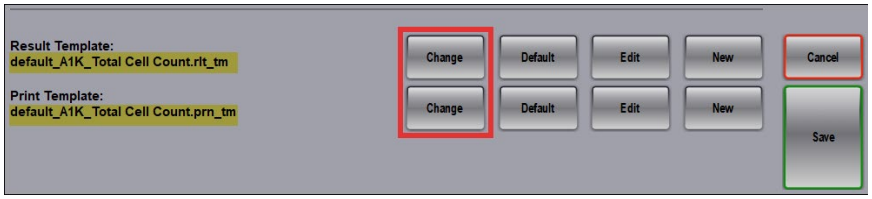


4. When the new template is complete, click the **Save** button.

5. Enter a **File Name** and save it to the default Auto 1000 *Template* folder by clicking the **Save** button. If the new template should *not* be associated with the current assay, you will need to change the Result Template.

Changing Result Templates

1. Return to the Imaging Mode screen and tap the **Change** button for either the *Result Template* or *Print Template*. Note the name of the template file currently associated with the assay.

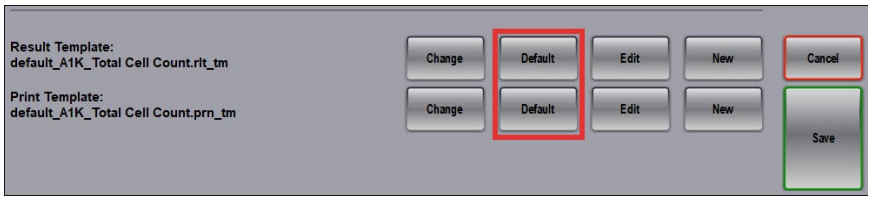


2. In the Select New Result Template screen, select a template file to be associated with the assay by tapping on a file icon in the default Auto 1000 Template folder or navigate to a file on an available drive. *Only files with an extension for the template type selected (i.e., .rlt_tm for Result Templates or .prn_tm for Print Templates) will be displayed in the Select file area of this screen.*

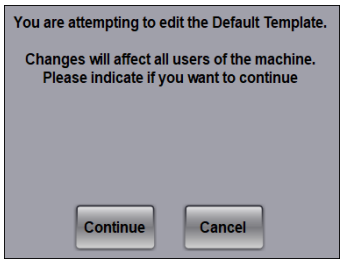
3. Confirm the name of the template file associated with the assay has changed per your selection and click the **Save** button to return to the Assay Parameters screen.

Restoring Default Templates

If the *Result Template* or *Print Template* files used as instrument defaults have been modified, select the **Default** button at the bottom of the Imaging Mode screen when the file is selected to restore it to the default version.



If the default *Results Template* is selected when you click the **Edit** button to modify the template, the following dialog is displayed. Click **Continue** *only* if you are comfortable modifying a template that will affect all users.

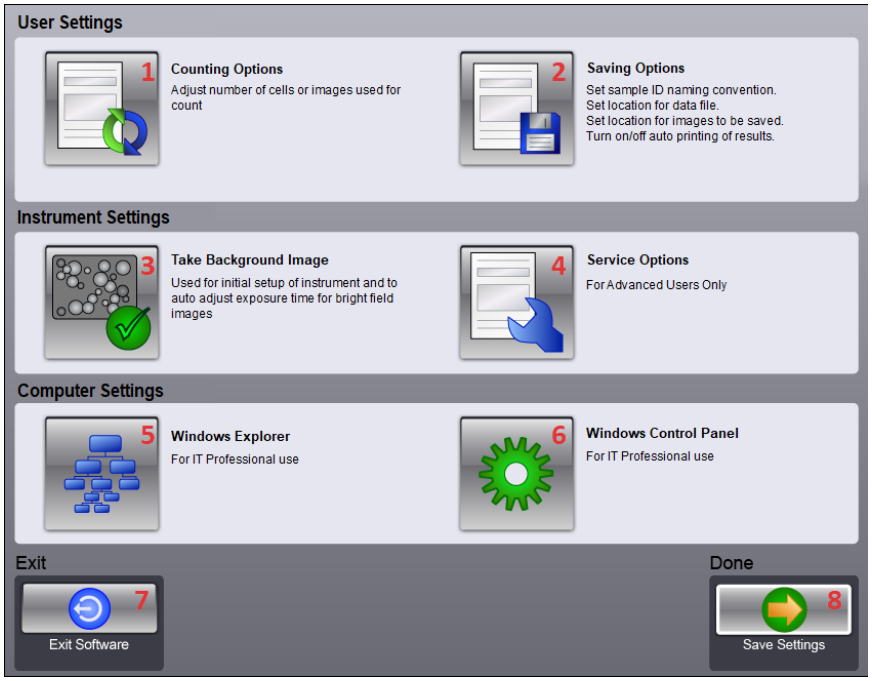


This page intentionally left blank.

Chapter 11. User and Instrument Settings

This chapter describes Auto 1000 *Counting Options* and *Saving Options* user settings. See *Taking a Background Image* on page *13* for instructions on using the *Take Background Image* instrument setting.

Tap the **Settings** icon from the Home screen to manage user and instrument settings. Note that some settings in this screen are intended for use only by Nexcelom Support or your IT team. For example, the *Service Options* instrument setting is intended *For Advanced Users Only*, while the *Windows Explorer* and *Windows Control Panel* computer settings are *For IT Professional Use*.



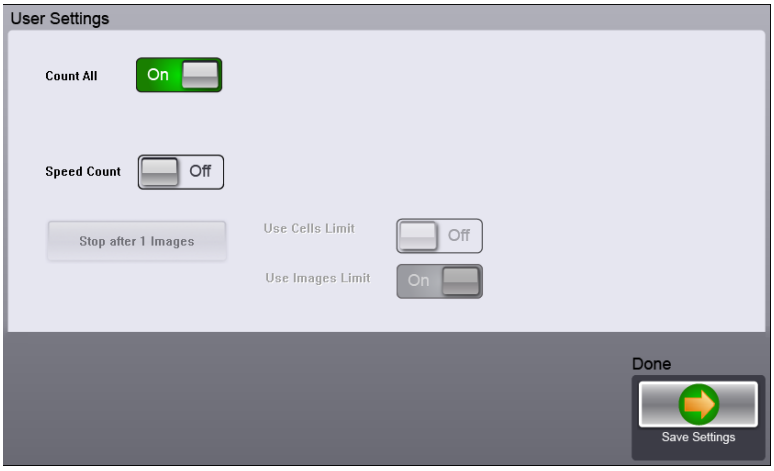
1. **Counting Options** – Allows users to specify counting options (*Count All* or *Speed Count* by limiting the number of cells or images).
2. **Saving Options** – Allows users to modify save options such as Sample ID naming convention, set default locations for data and image files, and enable/disable the *Auto Save* and *Auto Print* features.
3. **Take Background Image** – Allows users to take a background image the first time the instrument is used or if the system is moved to a new location. See *Taking a Background Image* on page *13*.
4. **Service Options** – Hardware settings that are password protected for use *only* by Nexcelom Support.
5. **Windows Explorer** – Allows access to Windows Explorer. *For IT Professional Use.*
6. **Windows Control Panel** – Allows access to Windows Control Panel. *For IT Professional Use.*
7. **Exit** – Closes the Cellometer Auto 1000 software and returns user to Windows desktop.
8. **Done** – Saves any changes to user, instrument and computer settings and returns to the Home screen.

COUNTING OPTIONS

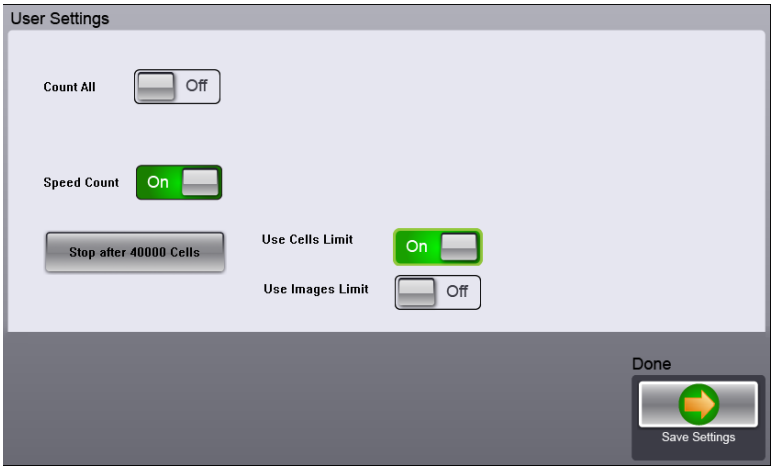
Tap the **Counting Options** icon on the Instrument Settings screen to modify counting options. You can select either the *Count All* or *Speed Count* option in the User Settings screen.

Note: Multiple images are taken of a sample during the counting process and are accessible when using the *Current Image Control* as images *A*, *B*, *C* and *D* (representing counting chamber fields of view). See the *Count Details Screen* on page *20* for details on switching between the display of these images in the viewing pane.

The **Count All** option is enabled by default. This option counts *all* cells in *all* images of the sample.



To enable speed counting, tap the **Speed Count** option and choose a parameter to limit the count: *Use Cells Limit* or *Use Images Limit*. Tap on the **Stop after…** button and use the virtual keypad to enter a number of cells or images to stop the counting process accordingly.

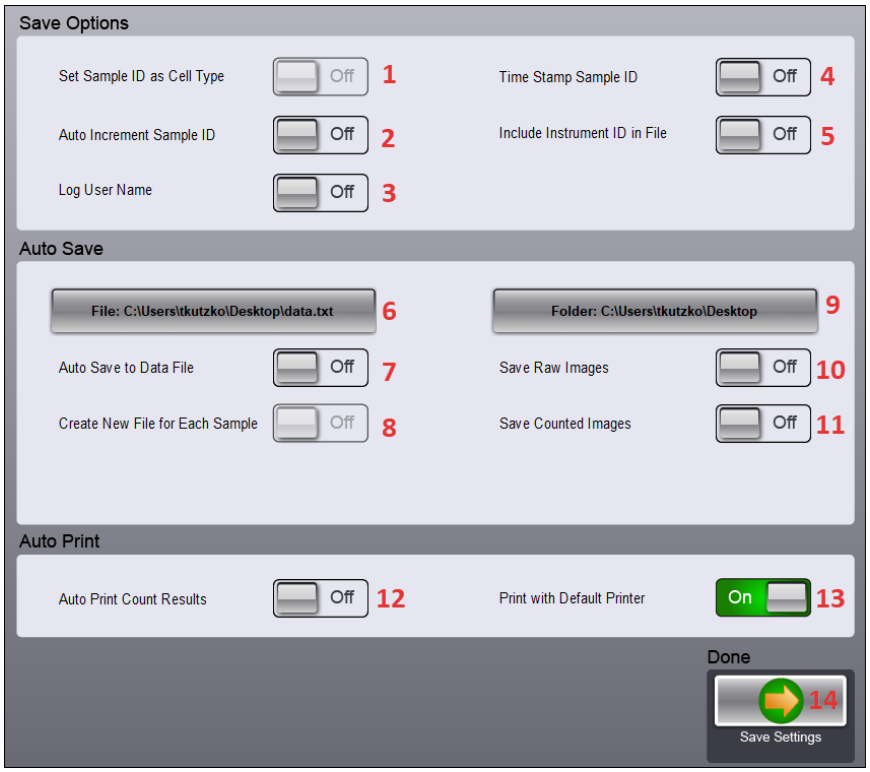


Note: The *Use Cells Limit* parameter defines maximum number of cells to be counted in an image before moving on to the next image, whereas the *Use Images Limit* parameter defines the number of images to be counted (e.g., if a value of *2* is entered, only cells in the first two images will be counted). *All four images of the sample will be taken regardless of Counting Options selected.*

Tap **Done** to save the current *Counting Options* user settings and return to the Instrument Settings screen.

SAVING OPTIONS

Tap the **Saving Options** icon on the Instrument Settings screen to modify save options such as Sample ID naming convention, set default locations for data and image files, and enable/disable the *Auto Save* and *Auto Print* features.



Save Options

1 **Set Sample ID as Cell Type** – Inputs a Sample ID default that matches the cell type being counted (e.g., *"Pan 02"* will be displayed for the *Pan-2* cell type). *This parameter cannot be selected until the* ***Auto Increment Sample ID*** *is enabled to allow automatic incrementing of Sample IDs.*

2 **Auto Increment Sample ID** – Appends the Sample ID default with an incremented numerical value (e.g., *Pan 02_001*, *Pan 02_002*, etc.).

3 **Log User Name** – Requires entry of a User ID as well as a Sample ID to be saved with the data. *Appends a User ID column to the data text file. Once a User ID is entered, it will remain as the default until changed.*

4 **Time Stamp Sample ID** – Appends Sample ID with a date/time stamp of when count was performed.

5 **Include Instrument ID in File** – Saves Instrument ID on which the count was performed with count results. *Appends an Instrument column to the data text file.*

Auto Save Options

6 **File:** *file_name.txt* **Button** – Allows users to specify the file path and name of the text file to be used for saving count result data.

7 **Auto Save to Data File** – Enables automatically saving of data to text file after a count is performed.

8 **Create New File for Each Sample** – *Requires **Auto Save to Data File** to be enabled.* Creates a new data text file each time cells are counted.

9 **Folder:** *file_path* **Button** – Allows users to specify the folder to be used for saving image files captured during a count. *It is recommended that image files be stored on the network or USB drive.*

10 **Save Raw Images** – Enables automatic saving of raw images (.pngs) to the folder specified. *Only raw images can be loaded into the Auto 1000 for reanalysis or sent to Nexcelom Support when requesting assistance with optimization of assay/cell type parameters.*

11 **Save Counted Images** – Enables automatic saving of counted images (.jpgs) to the folder specified. *Selecting this option saves two copies of each image; one image with the counted graphic overlay (i.e., "Counted" appears in the file name) while the other image displays cells without the graphic overlay.*

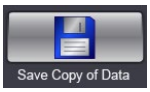
Auto Print Options

12 **Auto Print Results** – Enables automatic printing of count results after completion of each count. *If a printer is not connected to the instrument, you must also enable the **Print with Default Printer** option.*

13 **Print with Default Printer** – Enables use of the Windows default printer when automatically printing count results.

14 Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Defining Default Data File/Image Folder Locations



Users can save count results as necessary by tapping the **Save Copy of Data** icon on the Count Details screen. Follow these steps to define the default location for data and image files.

Note: The Auto Save feature does *not* need to be enabled in order to define default locations to be used when saving count result data and image files while viewing individual samples.

1. From the Home screen, tap the **Settings** icon.
2. In the *User Settings* area, tap the **Savings Options** icon.
3. In the *Auto Save* area of the screen, tap the **File:** *file_name.txt* button to specify the file path and text file to be used for saving count result data.
4. Navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**.
5. Select the **Folder:** *file_path* button to specify the folder to be used for saving image files captured during a count. *Due to the size of image files, it is recommended they be stored on the network or USB drive. Request help from your IT team before using the **Windows Explorer** option in the Computer Settings area to map available drives to your internal corporate network.*
6. Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Establishing a Windows Default Printer

Users can send count results to a printer as necessary by tapping the **Print** icon from the Count Results screen. Follow these steps to connect to a default Windows printer. *If a printer is connected to the instrument with a USB cable, establishing a default printer is not necessary.*

Note: The Auto Print feature does *not* need to be enabled in order to establish a Windows default printer to be used when sending count results to a printer while viewing individual samples.

1. From the Home screen, tap the **Settings** icon.
2. In the *User Settings* area, tap the **Savings Options** icon.
3. In the *Auto Print* area of the screen, tap the **Print with Default Printer** button to display as *ON*. *Request help from your IT team before using the **Windows Control Panel** option in the Computer Settings area to set up a Windows default printer.*
4. Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Setting Up Auto Save Feature

Follow these steps to set up automatic saving of count results each time you perform a count.

1. From the Home screen, tap the **Settings** icon.
2. In the *User Settings* area, tap the **Saving Options** icon.
3. In the *Auto Save* area of the Saving Options screen, tap the **Auto Save to Data File** button to display as *ON*.
4. Tap the **File:** *file_name.txt* button to specify the file path and text file to be used for saving count result data.

   Navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**.

   Note: File name entered will be used when the Auto Save feature is enabled *as well as* when manually saving count results using the **Save Copy of Data** icon on the Count Details screen.

5. To create a new file for each sample, tap the **Create New File for Each Sample** button to display as ON. *Keep in mind that selecting this option may result in a large volume of files.*
6. Select the **Folder:** *file_path* button to specify the folder to be used for saving image files captured during a count. *Due to the size of image files, it is recommended they be stored on the network or USB drive. Request help from your IT team before using the **Windows Explorer** option in the Computer Settings area to map available drives to your internal corporate network.*

   Navigate to a network location by selecting an available drive (displayed in bottom left corner of the screen) and tapping folder icons. Choose an existing file or tap **New File** to create a new file. *If necessary, create a new folder before creating a file.* Use the virtual keyboard to enter a file name and tap **Done**.

Note: Folder name entered will be used when the Auto Save feature is enabled *as well as* when manually saving count results using the **Save Copy of Data** icon on the Count Details screen.

7. Indicate the type of image files to be saved automatically by tapping the **Save Raw Images** and/or **Save Counted Images** buttons to display as *ON*. *Only raw images can be loaded into the Auto 1000 for reanalysis or sent to Nexcelom Support when requesting assistance with optimization of assay/cell type parameters. Selecting **Counted Images** saves two copies of each image; one image with the counted graphic overlay (i.e., "Counted" appears in the file name) while the other image displays cells without the graphic overlay.*

8. Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Setting Up Auto Print Feature

Follow these steps to set up automatic printing of count results each time you perform a count.

1. From the Home screen, tap the **Settings** icon.

2. In the *User Settings* area, tap the **Savings Options** icon.

3. In the *Auto Print* area of the screen, tap the **Auto Print Count Results** button to display as *ON*.
This enables automatic printing of count results to a printer connected to the instrument with USB a cable.

4. To enable printing to the Windows default printer on your network, tap the **Print with Default Printer** button to display as *ON*. *Request help from your IT team before using the **Windows Control Panel** option in the Computer Settings area to set up a Windows default printer.*

   Note: If **Print with Default Printer** is enabled, count results will be sent to the Windows default printer when the Auto Print feature is enabled *as well as* when manually printing count results using the **Print** icon on the Count Results screen.

5. Tap **Done** to save current *Saving Options* user settings and return to the Instrument Settings screen.

Chapter 12. Cleaning, Maintenance and Storage

Keeping the Auto 1000 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.

Note: If using the Auto 1000 within a biosafety cabinet, cleaning may not be required, or agents and materials may be adapted according to BSC system requirements. Please follow all the instructions provided by the manufacturer.

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

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

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

ROUTINE MAINTENANCE

No one other than Nexcelom-authorized personnel may service inside the protective instrument cover of the Cellometer Auto 1000. 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

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

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 *12*).
- DO NOT put anything heavier than ≥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 13. Troubleshooting and FAQs

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

TROUBLESHOOTING AND INSTRUMENT 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 *13*.

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 1000 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 instrument and repeat the task to be performed.

1. Power OFF the instrument.
2. Remove all USB devices.
3. Unplug the Power Cord from the outlet (wait 5 seconds).
4. Plug the Power Cord into the outlet.
5. Power the instrument ON (with no USB devices attached).

If the problem persists, contact Nexcelom Support for assistance at +1 (978) 327-5340 or email: 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 instrument, 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

FREQUENTLY ASKED QUESTIONS

Can SD025 slides be used on the Auto 1000?

No, SD025 slides are intended for use with small cells (such as bacteria) in higher magnification systems.

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

Contact support@nexcelom.com with the serial number of the instrument to request an upgrade.

The Power Cord for the instrument has been lost.

Contact support@nexcelom.com with the serial number of the instrument to request a replacement.

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 *48* 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 14. Nexcelom Support

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/ 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

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

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

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

REPORTING AN ISSUE TO SUPPORT

If an issue encountered *cannot* be resolved using troubleshooting steps presented in this guide (see *Troubleshooting and Instrument Messages* on page *65*) or the issue persists after rebooting the instrument, perform the following steps before creating a Support ticket:

1. Record the instrument or software error message.
2. Record the sequence of events that caused the issue, if possible.
3. Close the 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

Appendix A. Consumables

This appendix presents Nexcelom consumables designed specifically for the Auto 1000 such as disposable counting chamber slides and counting beads. Catalog numbers are listed for all available sizes.

COUNTING CHAMBER SLIDES

Cellometer *Counting Chamber Slides* are compatible with all Cellometer systems. Each all plastic, disposable slide contains two sample counting chambers with precisely controlled height. The fixed 20 µL 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. *Peeled and ready to use.*	Box of 100 slides for 200 counts	1 Box	
CHT4-PD100-003	Standard chamber thickness. Packed in microscope slide boxes. *Peeled and ready to use.*	Case of 500 slides for 1,000 counts (10 individual boxes)	1 Case	
CHT4-PD100-303	Standard chamber thickness. Packed in microscope slide boxes. *Peeled and ready to use.*	Case of 1,500 slides for 3,000 counts (30 individual boxes)	1 Case	
CHT4-PD100-503	Standard chamber thickness. Packed in microscope slide boxes. *Peeled and ready to use.*	Case of 2,500 slides for 5,000 counts (50 individual boxes)	1 Case	

Catalog #	Description	Size	Unit	Image
CHT4-SD100-002	Standard chamber thickness. Packed with protective film on both sides. *Remove protective film before use.*	Box of 75 slides good for 150 counts	1 Box	
CHT4-SD100-014	Standard chamber thickness. Packed with protective film on both sides. *Remove protective film before use.*	Case of 900 slides good for 1,800 counts	1 Case	
CHT4-SD100-314	Standard chamber thickness. Packed with protective film on both sides. *Remove protective film before use.*	Case of 2,700 slides good for 5,400 counts	1 Case	
CHT4-SD100-514	Standard chamber thickness. Packed with protective film on both sides. *Remove protective film before use.*	Case of 4,500 slides good for 9,000 counts	1 Case	

Visit the Nexcelom-products/cellometer-disposable-counting-chambers page on our website for an up-to-date list and to purchase Cellometer counting chamber slides directly from Nexcelom Bioscience.

COUNTING BEADS

Cellometer *Counting Beads* may be used to verify instrument functionality and establish routine quality control SOPs for daily, weekly or monthly instrument performance. Bead products are *not* intended to replace certification by *Installation/Operation Qualification (IQ/OQ)* procedures.

Catalog #	Description	Size	Unit	Image
B05-02-050	5-micron Polystyrene beads in a trypan blue solution; good for 200 counts. Concentration: 5 x 10^6 beads/mL	1 mL/Vial	4 Vials	
B10-02-020	10-micron Polystyrene beads in a trypan blue solution; good for 200 counts. Concentration: 2 x 10^6 beads/mL	1 mL/Vial	4 Vials	
B15-02-010	15-micron Polystyrene beads in a trypan blue solution; good for 200 counts. Concentration: 1 x 10^6 beads/mL	1 mL/Vial	4 Vials	

Visit the Nexcelom-products/counting-beads page on our website for an up-to-date list and to purchase Cellometer counting beads directly from Nexcelom Bioscience.

This page intentionally left blank.

Appendix B. Declaration of Conformity

The Cellometer® Auto 1000 Cell Counter conforms to the appropriate country standards and governing regulations as listed in the *Declaration of Conformity* provided by Nexcelom Bioscience LLC (manufacturer of Auto 1000 instruments), and complies with requirements described under Supplementary Information as presented on the following page.

Note: The *Declaration of Conformity* presented on the following page is current as of the date of publication for this user manual. If you have questions or would like to request if a more recent *Declaration of Conformity* version is available, contact Nexcelom Support at +1 (978) 327-5340 or email: support@nexcelom.com

Nexcelom Bioscience LLC
360 Merrimack Street
Building 9
Lawrence, MA 01843

Declaration of Conformity

According to EN 45014

Manufacturer:	Nexcelom Bioscience LLC
Address:	360 Merrimack Street, Building 9 Lawrence, MA 01843 USA
Product Name:	Cellometer Cell Counter
Model Number:	Auto 1000

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 61010-1:2001 Safety requirements for electrical equipment for measurement, control, and laboratory use
- EMC:
  - EN 55022:2006/A1:2007 Class A ITE emissions requirements (EU)
  - ICES-003 Issue 4 Class A Digital Apparatus emission requirements (Canada)
  - FCC 47 CFR Part 15 Class A emissions requirements (USA)
  - VCCI Class A ITE emissions requirements (Japan)
  - AS/NZS CISPR 22: 2006 Class A ITE emissions requirements (Australia)
  - EN 61326:2006 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 2006/95/EC and the EMC Directive 2004/108/EC, and carries the CE marking accordingly.

This product was tested in a prototype configuration as slight modifications for production have been made that will not affect the above conformity.

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.

Peter Y. Li

Manager, Regulatory Compliance

Appendix C. Warranty and License Details

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 1000 User Manual

8001505, Rev D

360 Merrimack St, Suite 200

Lawrence, MA 01843 USA

T: +1 (978) 327-5340

F: +1 (978) 327-5341

info@nexcelom.com

www.nexcelom.com

CE