peqlab

Cellometer® Auto 2000

User Manual

Cellometer Auto 2000 User Manual

Introduction

What is Cellometer Auto 2000?

Cellometer® Auto 2000 is a compact, automated cell counting system utilizing dual fluorescence to detect, count, measure cell size and calculate cell concentration from a 20uL cell sample. The basic principle of the Cellometer automatic cell counter is imaging cytometry. Cells are loaded into the Disposable Counting Chamber and automatically spread into a thin layer by capillary action. Cellometer Auto 2000 then captures images of cells in the counting chamber, analyzes the number of cells, sizes and fluorescence intensity of each cell, and then converts this data into concentration, size and viability.

The Cellometer Auto 2000 system consists of 2 main components:

1. Cellometer Auto 2000 instrument with analyzing software
2. Disposable Counting Chambers

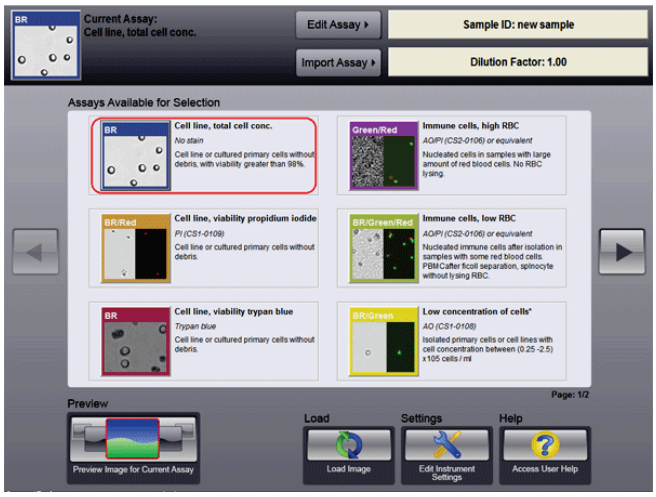
   Disposable counting chamber accommodates 2 individual samples and can be loaded through either port.

   Pipette 20uL of cell sample into one of the ports with any standard single channel pipette.

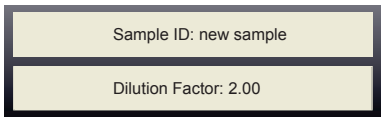
   Cellometer Auto 2000 comes with a starter set of 75 slides. Slides can be ordered directly from Nexcelom or your authorized Nexcelom dealer.

Quick Operation Instructions

1. Select an Assay



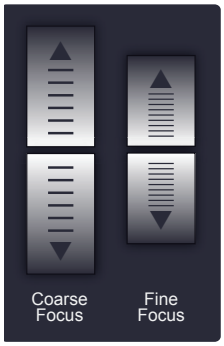
2. Input Sample ID and Dilution Factor



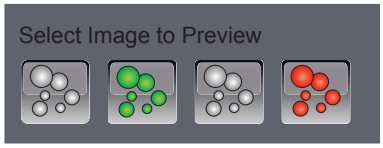
3. Prepare Sample, load disposable counting chamber and insert into instrument

4. Click "Preview"

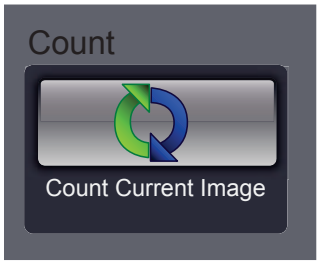
5. Adjust Focus



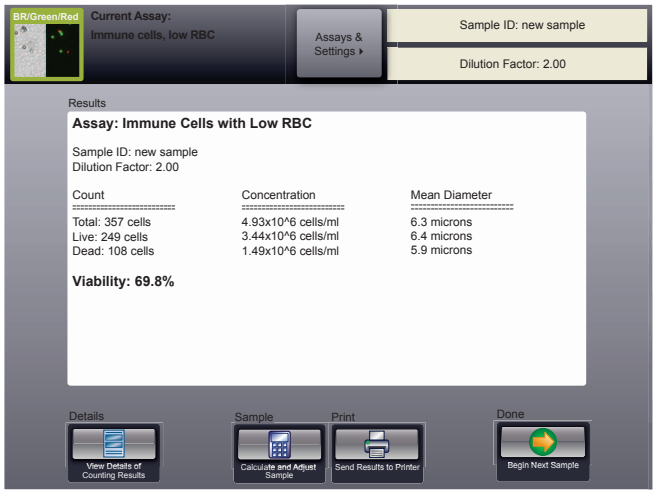
6. Select Green and Red images to preview and adjust exposure if needed



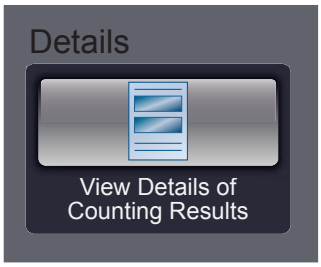
7. Click "Count" to begin counting process



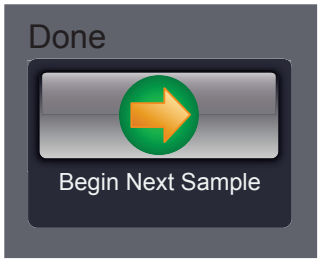
8 .Review Counting Results



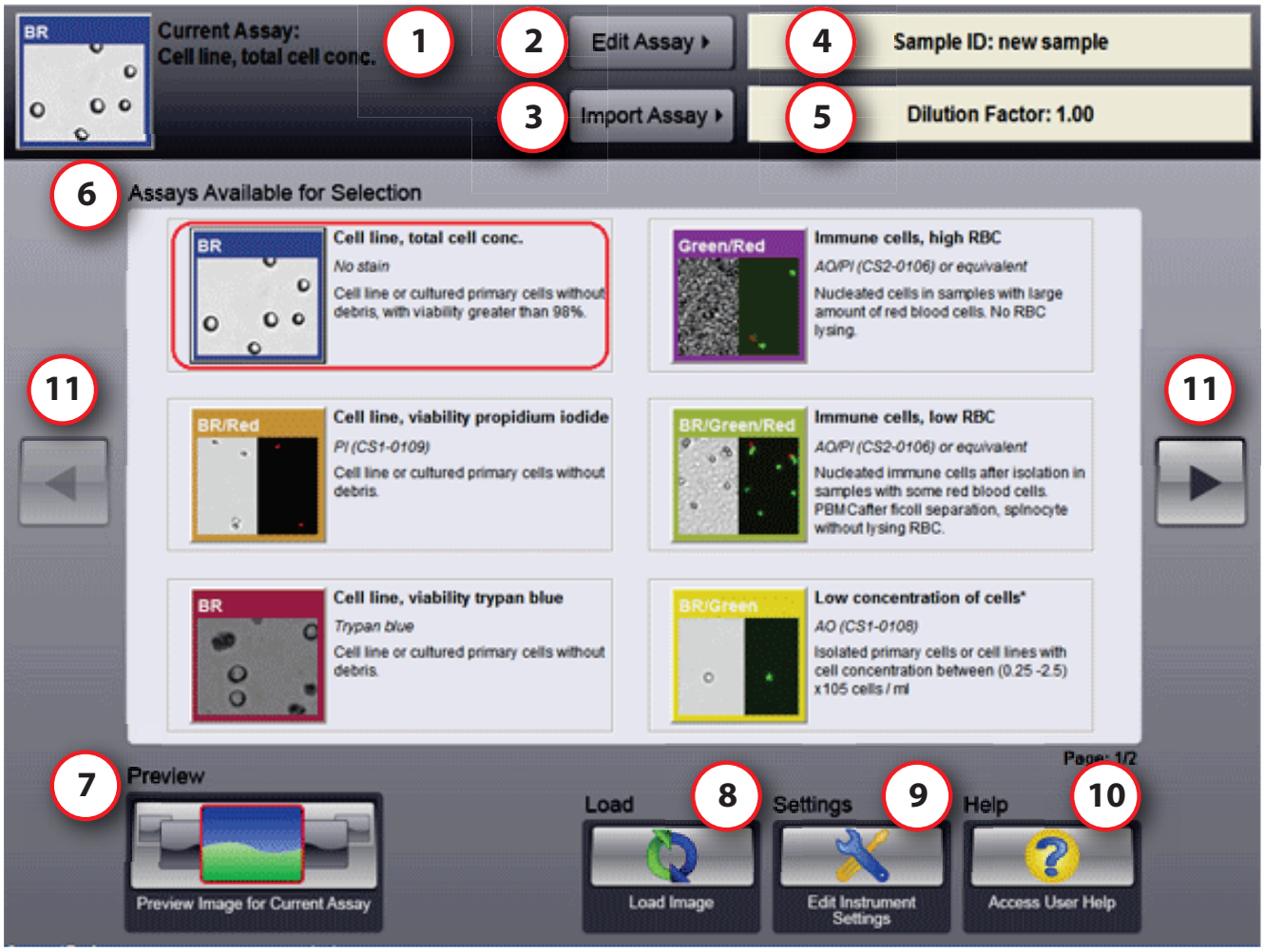
9. Select Details to review cell images and counted cell images



10. Select Next Sample or Assay and Settings when done

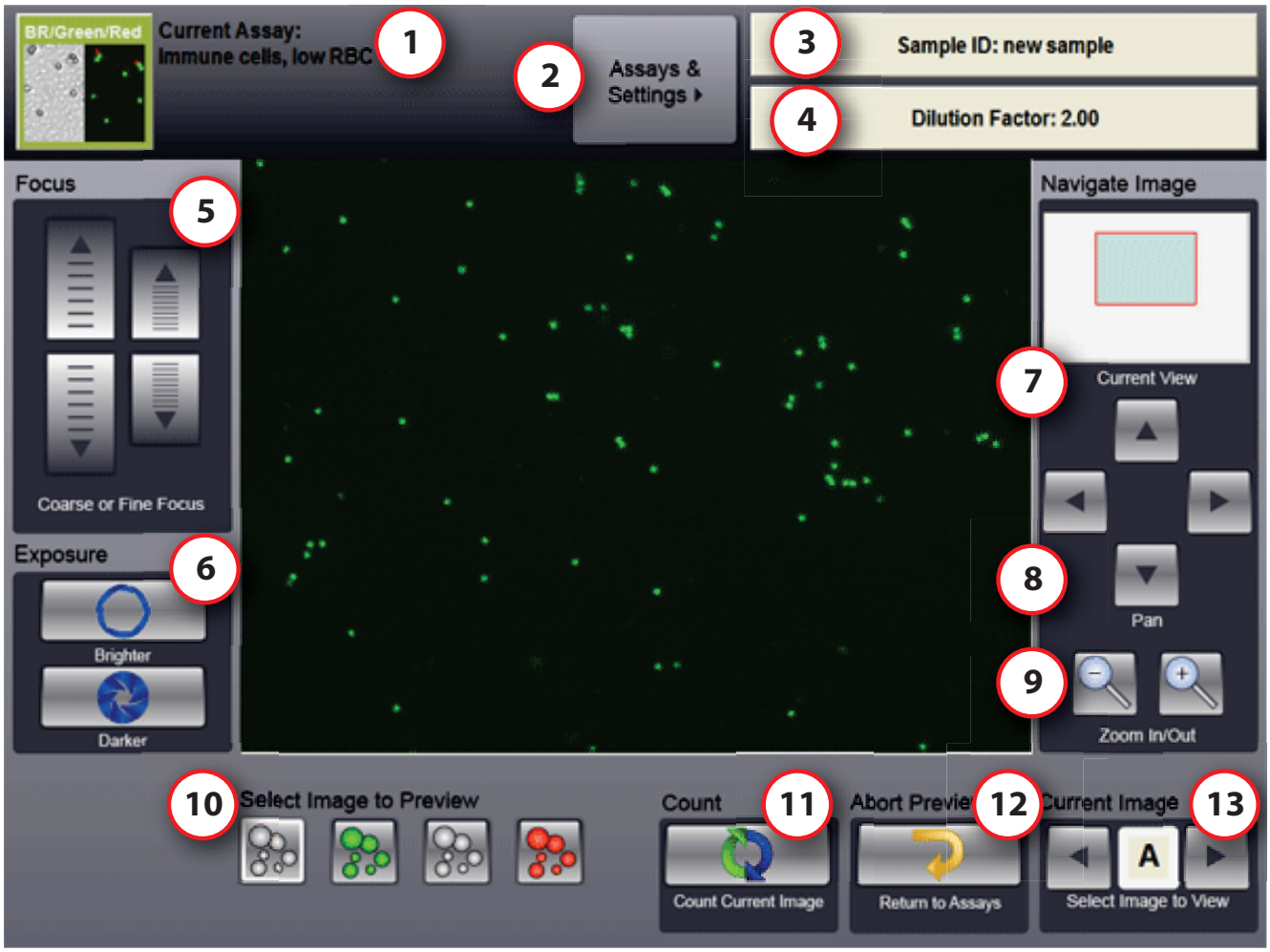


User Interface - Assay Selection Screen



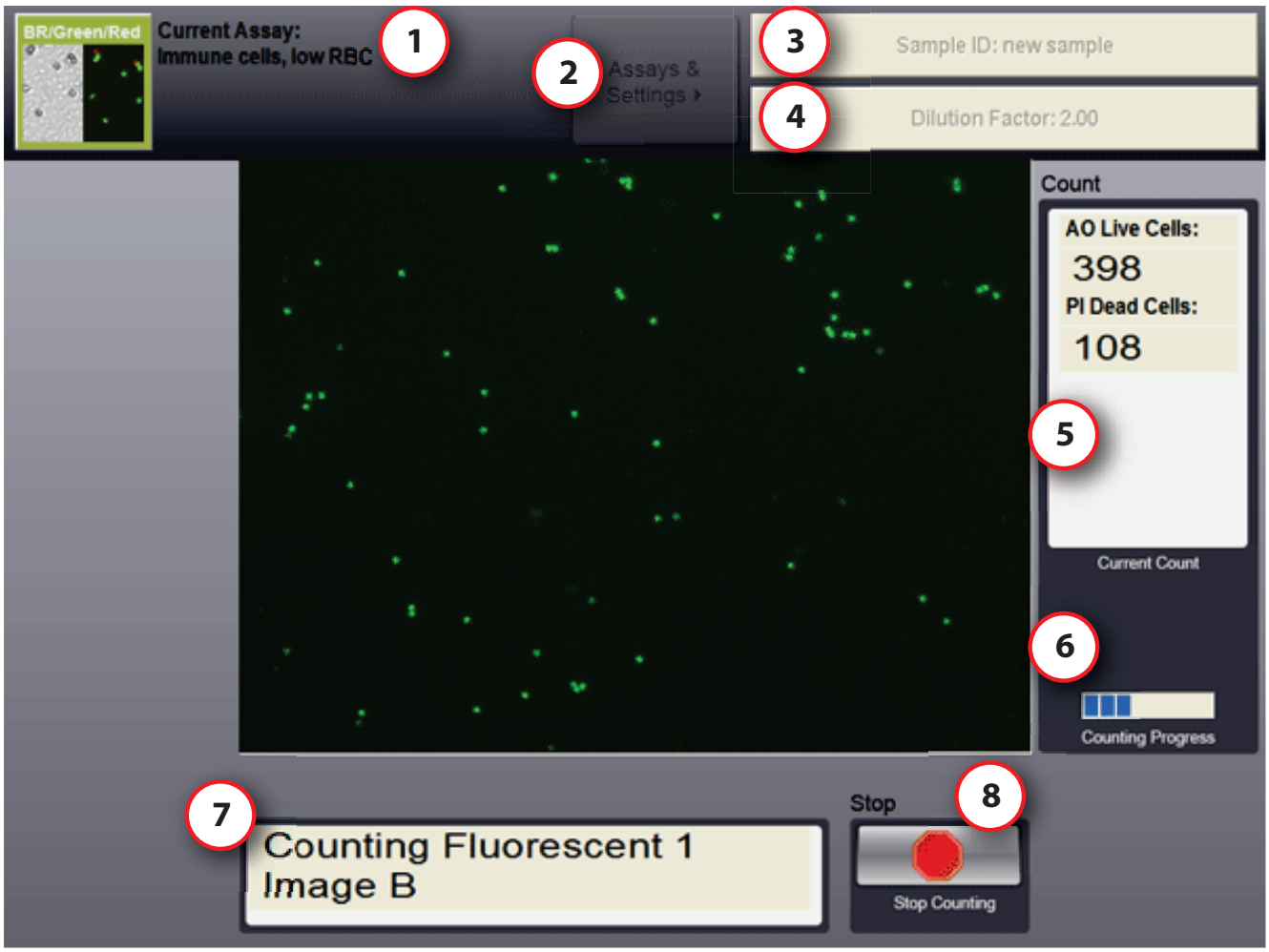
1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Edit Assay** Edit the Assay settings
3. **Import Assay** Import additional assays
4. **Sample ID** Enter a Sample ID
5. **Dilution Factor** Enter a Dilution Factor
6. **Assays Available** List of Assays available for selection
7. **Preview** Preview the cell sample image
8. **Load** Load and analyze previously saved images
9. **Settings** Edit instrument and UI settings
10. **Help** Access help options
11. **Left/Right Arrows** Page left/right to access all assays available

Preview Screen



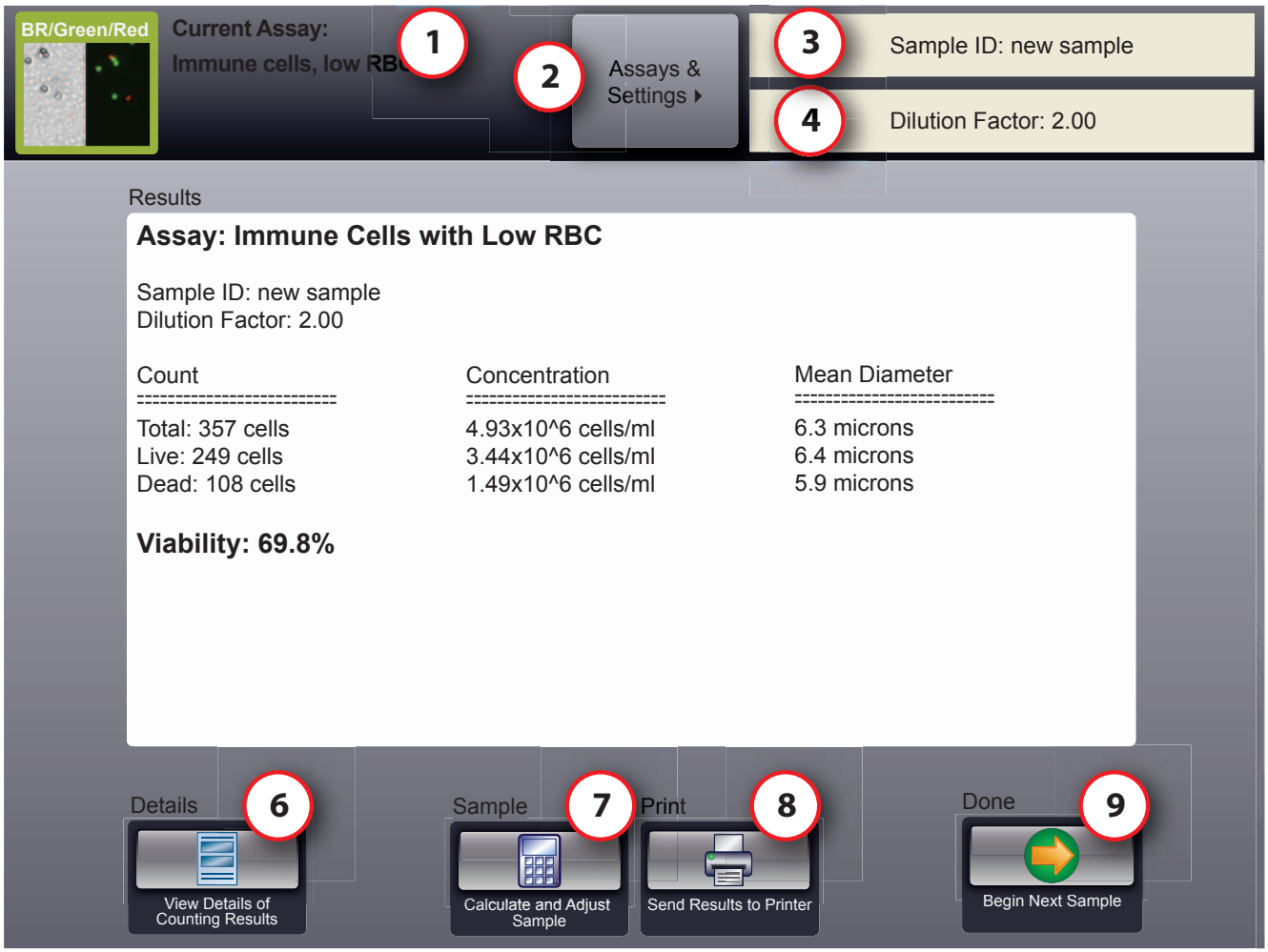
1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Assays & Settings** Return to Assay Selection Screen
3. **Sample ID** Enter a Sample ID
4. **Dilution Factor** Enter a Dilution Factor
5. **Focus** Fine and coarse focus adjustment of the current cell image
6. **Exposure** Adjust the exposure time of the current cell image
7. **Current View** Red rectangle indicates what area of the cell image is currently displayed
8. **Pan** Moves the area currently being shown within the current cell image
9. **Zoom** Zooms in or out of the current cell image
10. **Select Image to Preview** Select brightfield, green or red fluorescence images to preview
11. **Count** Starts counting of the current cell image
12. **Abort Preview** Cancels current cell preview and returns Assay Selection Screen
13. **Current Image** Select location on slide to preview

Counting Screen



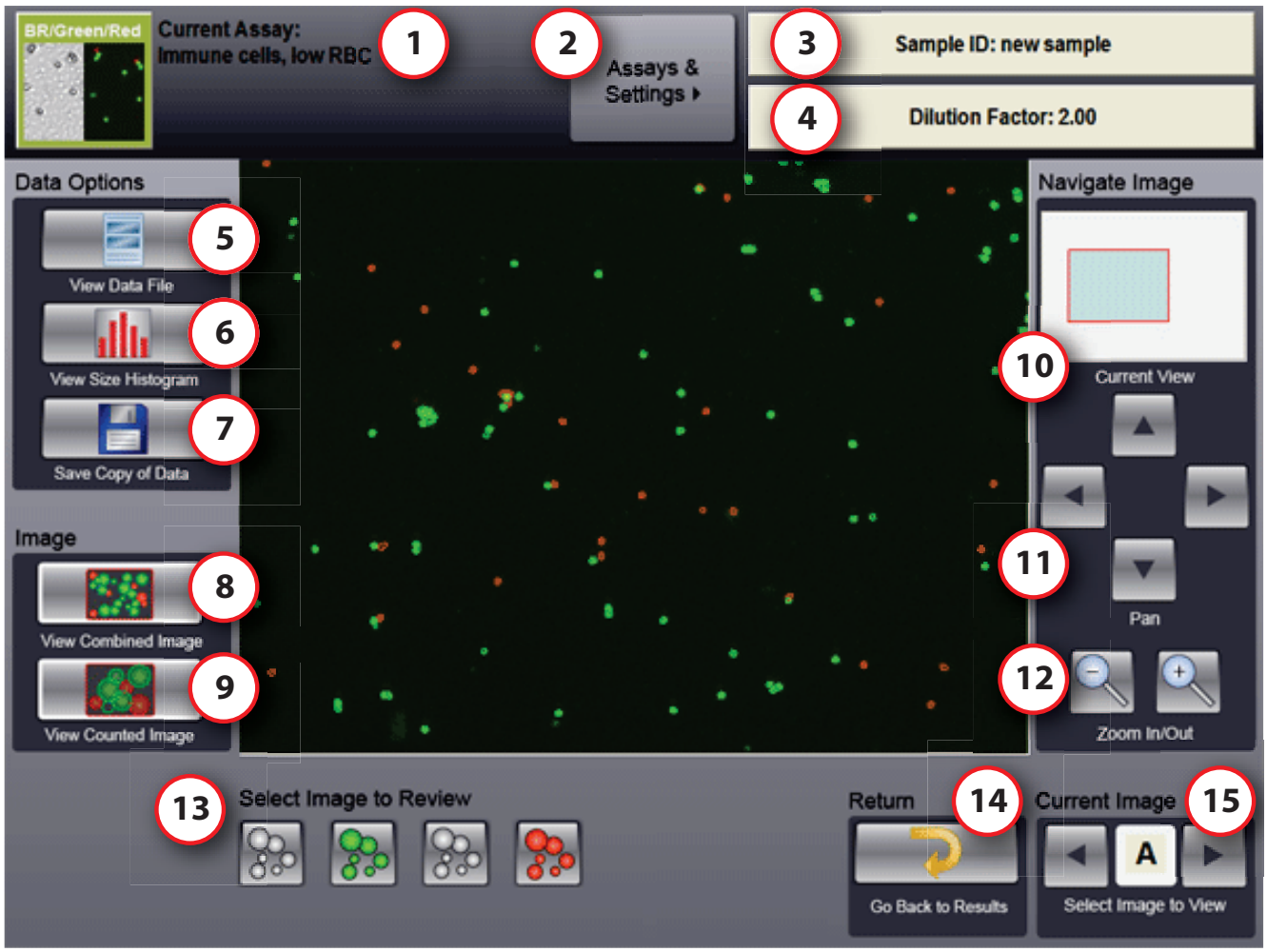
1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Assays & Settings** Return to Assay Selection Screen (disabled while count is in progress)
3. **Sample ID** Enter a Sample ID (disabled while count is in progress)
4. **Dilution Factor** Enter a Dilution Factor (disabled while count is in progress)
5. **Current Count** Shows cell count numbers
6. **Counting Progress** Visually indicates the current count progress
7. **Image Being Counted** Indicates which image is currently being counted
8. **Stop** Stops cell counting

Results Screen



1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Assays & Settings** Return to Assay Selection Screen
3. **Sample ID** Enter a Sample ID
4. **Dilution Factor** Enter a Dilution Factor
5. **Results** Displays cell counting results including total cell count, live/dead cell count and viability
6. **Details** Go to the Details Screen
7. **Sample Adjustment Calculator** Launch the sample adjustment calculator. Useful for sample adjustment to get desired concentration or total cell number.
8. **Print** Send the counting results to a network printer
9. **Done** Start a preview of a new cell sample using the same Assay settings

Details Screen



1. **Current Assay** Indicates which Assay is currently selected to analyze a cell sample
2. **Assays & Settings** Return to Assay Selection Screen
3. **Sample ID** Enter a Sample ID
4. **Dilution Factor** Enter a Dilution Factor
5. **View Data File** Opens data file to view or print
6. **View Size Histogram** Opens separate window to display cell size histogram
7. **Save Copy of Data** Saves the current counting results to the data file
8. **View Combined Image** Cell image showing F1 and F2 fluorescent objects in a single merged image (available only when an assay uses both F1 and F2 images).
9. **View Counted Image** Shows cell image with green and red outlines to indicate cells counted
10. **Current View** Red rectangle indicates what area of the cell image is currently displayed
11. **Pan** Moves the area currently being shown within the current cell image
12. **Zoom** Zooms in or out of the current cell image
13. **Select Image to Review** View the brightfield, Green or red fluorescence counted images
14. **Return** Return to Results Screen
15. **Current Image** Select location on slide to view counted image

Getting Started

Setting up Cellometer Auto 2000

All Nexcelom products undergo a rigorous quality inspection prior to shipment and all reasonable precautions are taken in preparing them for shipment to assure safe delivery.

The instrument should be unpacked and inspected for mechanical damage upon receipt. Mechanical inspection involves checking for signs of physical damage.

If damage is apparent, or any components are missing, please immediately contact Nexcelom (+1-978-327-5340 or support@nexcelom.com) or your local dealer.

After unpacking the instrument, plug the Cellometer Power Cable into the back of the instrument. The Auto 2000 is pre-configured with the Cellometer Auto 2000 software. No additional setup or configuration is required.

Tutorials

Overview

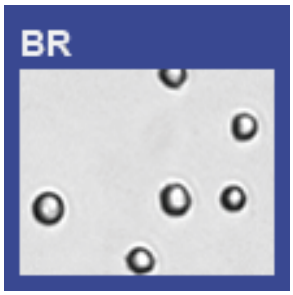
The following tutorials are intended as a guide to performing various cell counting assays using the Auto 2000. General sample preparation hints are included for each tutorial as well as instrument and software operation instructions.

Each of the assays can also be performed using the sample images included in the software as a demonstration of the Auto 2000. Sample images for each cell counting assay can be found at C:\ Program Files\Nexcelom\Assay_Images\

Staining Solutions

Trypan Blue Stock solution: 0.2% in PBS. Use 1:1 with cell sample. Dilution factor: **2**
AO (CS1-0108) Stock solution: 10ug/mL in PBS. Use 1:1 with cell sample. Dilution factor: **2**
PI (CS1-0109) Stock solution: 100ug/mL in PBS. Use 1:1 with cell sample. Dilution factor: **2**
AO/PI (CS2-0160) Stock solution: 5ug/mL AO; 100ug/mL PI in PBS. Use 1:1 with cell sample. Dilution factor: **2**

Installed Assays and Descriptions

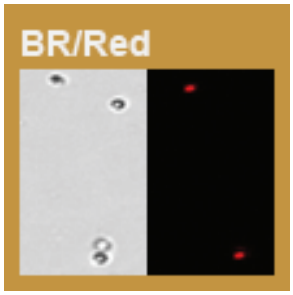


Cell Line, Total Cell Concentration

No Stain

Cell line or primary cells without debris, with viability greater than 98%.

After taking brightfield images of a cell sample, all cells are counted to determine total cell concentration.

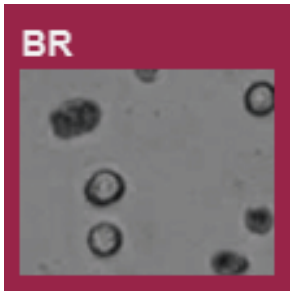


Cell Line, Viability Propidium Iodide

PI (CS1-0109)

Cell line or cultured primary cells without debris.

Propidium iodide is routinely used to determine cell viability. PI is a fluorescent stain that only penetrates dead cells and emits in the 'red' range (live cells are unaffected by PI). After taking both brightfield and 'red' fluorescent images of a PI stained sample, all cells (from brightfield channel) and dead cells (from 'red' fluorescent channel) are counted to determine total and dead cell concentrations and compute the percent viability.

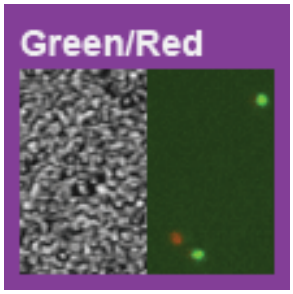


Cell Line, Viability Trypan Blue

Trypan Blue

Cell line or cultured primary cells without debris.

Trypan blue is routinely used to determine cell viability. Trypan blue penetrates and stains dead cells and leaves live cells unstained. After taking brightfield images of the stained sample, live and dead cells are counted to determine total, live and dead cell concentrations as well as compute percent viability.

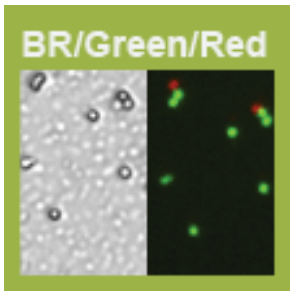


Immune Cells, High RBC

AO/PI (CS2-0160) or equivalent

Nucleated cells in samples with large amount of red blood cells. No RBC lysing.

Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide is a fluorescent stain that only penetrates dead cells and emits in the 'red' range. After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

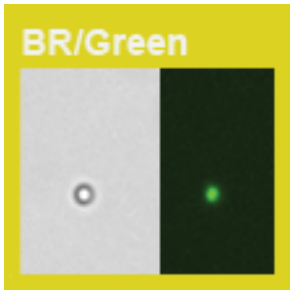


Immune Cells, Low RBC

AO/PI (CS2-0106) or equivalent

Nucleated immune cells after isolation in samples with some red blood cells. PBMC Cafter ficoll separation, splenocyte without lysing RBC.

Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide is a fluorescent stain that only penetrates dead cells and emits in the 'red' range. After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

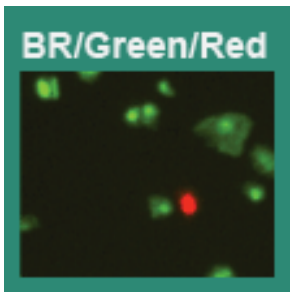


Low Concentration of Cells

AO (CS1-0108)

Isolated primary cells or cell lines with cell concentration between (0.25 - 2.5) x 10^5 cells/ml

Cell Samples are prepared with acridine orange, a nuclear stain that emits in the 'green' range. After taking 'green' fluorescent images, all fluorescent cells are counted and their concentration is determined. Brightfield images of the sample can be taken but are not used for counting.



Stem Cells, Primary Cells, Cell Lines

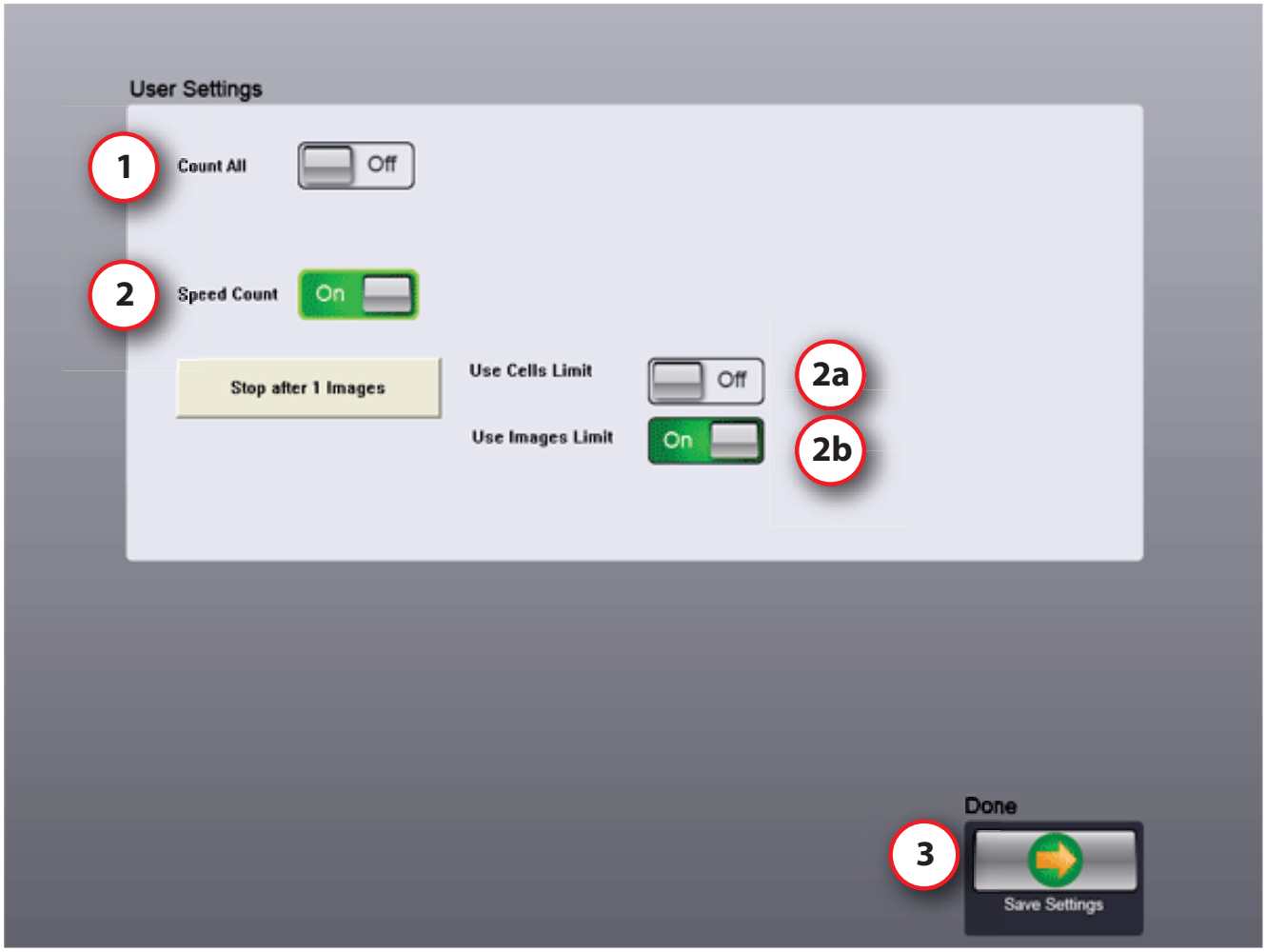
AO/PI ((CS2-0106) or equivalent

Stem cells, primary cells, cell lines, cell samples from dissociated tissues with debris.

Acridine orange is a nuclear stain that emits in the 'green' range and is used to stain live cells. Propidium iodide is a fluorescent stain that only penetrates dead cells and emits in the 'red' range. After taking both 'green' and 'red' fluorescent images, all fluorescent cells in each channel are counted and the concentration of live ('green' fluorescent) and dead ('red' fluorescent) cells as well as viability are determined.

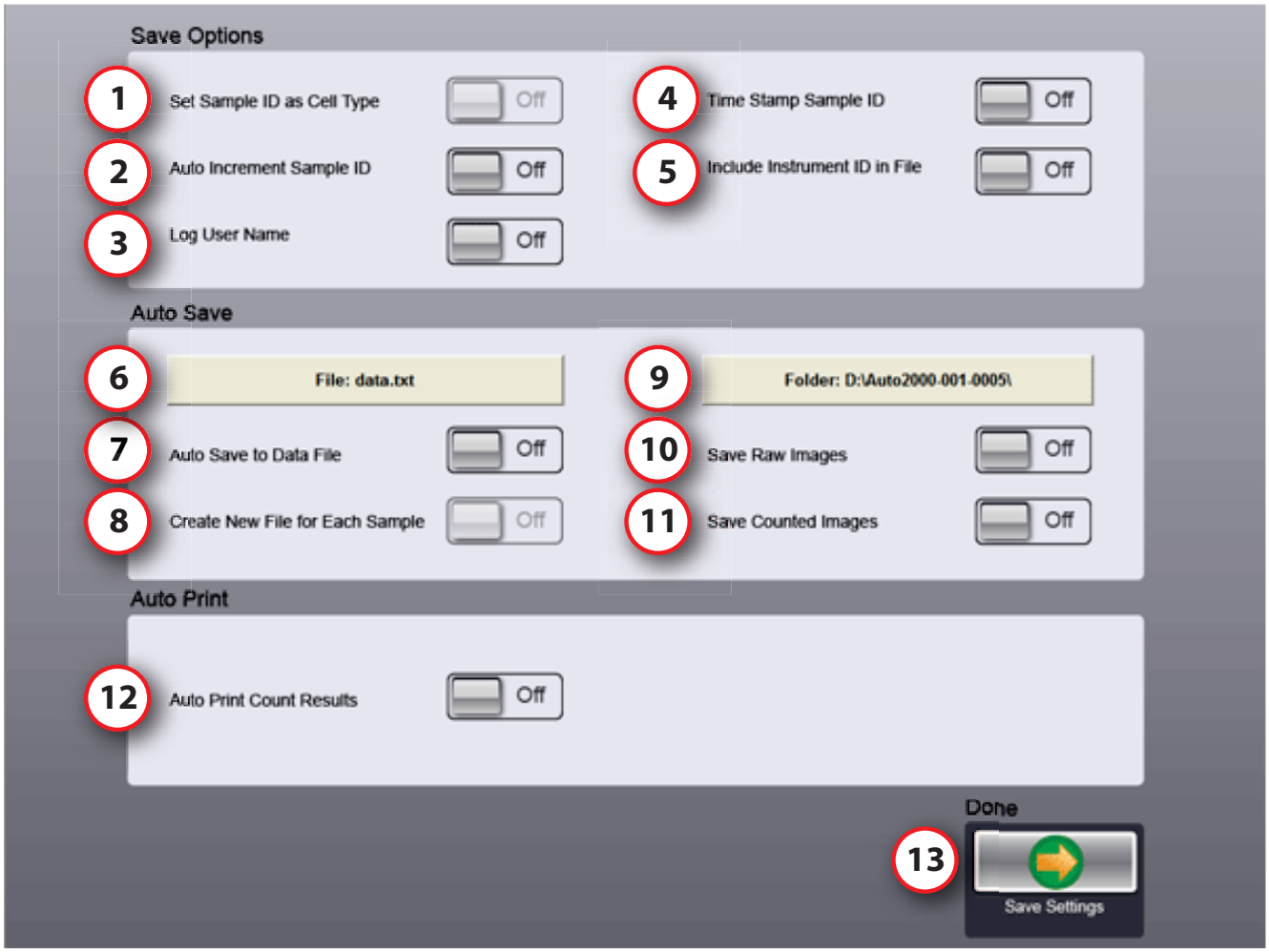
Operation Reference

Counting Options



1. **Count All** Use all 4 positions of slide used for cell images
2. **Speed Count** Have counting use Cell Limit or Image Limit
   a. **Use Cells Limit** Check to stop counting after # of user defined cells are counted and the next frame has finished counting
   b. **Use Images Limit** Stop counting after finishing user defined images that are less than 4 images
3. **Done: Save Settings** Saves the current settings and returns to previous screen.

Saving Options



Save Options

1. **Set Sample ID as Cell Type** Automatically input the Sample ID to match the Cell Type parameter name being used for counting
2. **Auto Increment Sample ID** Automatically append the Sample ID with an incremental numerical value (Example: CHO sample_001)
3. **Log User Name** Require the user to enter in an ID that will be recorded with the data
4. **Time Stamp Sample ID** Automatically append the Sample ID with the date and time the count was performed
5. **Include Instrument ID in File** Automatically append the Instrument ID to the sample ID after the count is performed

Auto Save

6. **File** File Dialog Window which allows the user to specify where the data.txt file will be saved when Auto Save to Data File is selected.
7. **Auto Save to Data File** Automatically save the data into the Data.txt file after a count is performed
8. **Create New File for Each Sample** Automatically create a new data.txt file for each sample and save the counting results to it after a count is performed
9. **Folder** Opens File Dialog Window which allows the user to specify where the image folder will be saved when Save Raw Images or Save Counted Images is selected.

10. **Save Raw Images** Automatically saves the raw images to the folder specified using the Folder command
11. **Save Counted Images** Automatically saves the counted images to the folder specified using the Folder command

Auto Print

12. **Auto Print Results** Send to the default printer upon completion of count.
13. **Done: Save Settings** Save new settings and return to Assay and Settings main screen.

Technical Information

Specifications

Size and weight	Height 13.4 inches (340 mm) Width 11.1 inches (283 mm) Depth 12.8 inches (324 mm) Weight 26.0 Pounds (11.8 kg)
Environmental Requirements	Typical biology lab environment
Display	10.4 inch, 1024 X 768 TFT with LED back light
Touch screen	4-Pin resistive with controller 10.4 inch format
Processor	Intel® Atom
Memory	RAM Memory: 2 GB DDR2 Hard drive size: 8 GB compact flash BIOS: 2 MB flash
Operating system	Window 7 embedded system
External Connectors	1 USB 2.0 1 Ethernet 1 Power input plug
Power input	12 VDC @ 5.0 Amps
External button	On/off 2-color button Orange: Standby Blue: Ready to Count
Optics	Excitation Channel #1: 470 nm Channel #2: 525 nm Emission Channel #1: 535 nm Channel #2: 660 nm Objective: 4X plan achromat
Camera	½ inch interline 1.4 MP monochrome Pixel size: 4.65 uM square

Getting Support

We provide free consulting for your lab on cell type, assay type and sample conditions. The goal is to find the most suitable cell counting / analysis solution for your lab.

Our team of Application Specialists are trained biologists with comprehensive understandings of cell counting, viability assay methods and best practices.

Please contact us when you need to discuss specific types of cells, sample conditions, and applications. We are available between 8:30am and 5:00pm Eastern US time. For immediate help, please call us at 1-978-327-5340 or email

support@nexcelom.com for applications and other technical information
sales@nexcelom.com for placing a purchase order or inquiries on a purchase order
info@nexcelom.com for general inquiries

Warranty Information

Nexcelom warrants that Nexcelom instrumentation products shall, for a period of 12 (twelve) 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 manufacturers 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.

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. A valid Return Material Authorization Number (RMA#) is required for all warranty repairs. For RMA instructions, please contact our customer service department at 978-327-5340 or email support@nexcelom.com.

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Document 8001334 Rev A