



STEM CELL LABORATORY (STCL)



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Operation of Cellometer Auto 2000

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STCL-EQUIP-022

OPERATION OF CELLOMETER AUTO 2000

1 PURPOSE

- 1.1 This procedure outlines the operation and maintenance of the Cellometer Auto 2000 in the Stem Cell Laboratory.

2 INTRODUCTION

- 2.1 The Cellometer Auto 2000 is an automated cell counting system that uses a bright field plus dual fluorescence to detect, count, measure cell size and calculate cell concentration and cell viability.
- 2.2 The bright field is used to differentiate cells from debris and decluster small clumps when appropriate. Large clumps of cells will be counted as debris.
- 2.3 Acridine Orange (AO) and Propidium Iodide (PI) are nucleic acid binding dyes which produce strong fluorescence after binding to DNA. AOPI is pH sensitive.
 - 2.3.1 AO is permeable to both live and dead cells and will enter the cell membrane to bind to its DNA. Under blue excitation, green fluorescence is emitted and detected for calculation of total cell counts.
 - 2.3.2 PI is only permeable to dead cells. Both AO and PI will enter a dead cell through the damaged cell membrane and bind to its DNA. PI enters the damaged cell membrane and binds to its DNA and will emit a strong red signal used to calculate dead cell counts. Under blue excitation, the green fluorescence by AO is absorbed by PI producing an extremely weak green signal.
 - 2.3.3 Total live cells are calculated by the instrument by subtracting dead cells (red) from total cells (green).

3 SCOPE AND RESPONSIBILITIES

- 3.1 This document will be referred to for general use, maintenance, cleaning, and calibration of the Cellometer Auto 2000.
- 3.2 Stem Cell Laboratory personnel will use this procedure to operate and maintain the Cellometer Auto 2000.
- 3.3 Report all operational malfunctions to the appropriate staff for repair.
- 3.4 Operators should refer to the Cellometer Auto 2000 Instructions Manual for information regarding installation, proper use and operation, identification of internal parts, and troubleshooting.

4 DEFINITIONS/ACRONYMS

- 4.1 AOPI: Acridine Orange/Propidium Iodide
- 4.2 GMP: Good Manufacturing Practice
- 4.3 QC: Quality Control
- 4.4 COA: Certificate of Analysis

5 MATERIALS

- 5.1 AOPI Staining Solution
- 5.2 Plastic Disposable Cell Counter Chambers for Cellometer Auto 2000
- 5.3 Eppendorf Tube
 - 5.3.1 Only use polypropylene tubes or plates. Do not use polystyrene tubes or plates as cells may bind to the plastic and alter the count.
- 5.4 Pipette tips
- 5.5 1x DPBS Dulbecco's Phosphate-Buffered Saline
- 5.6 Cellometer Check Validation Bead Solution

6 EQUIPMENT

- 6.1 Cellometer Auto 2000
- 6.2 Pipetteman or micropipettor to deliver 20 to 200 μ L

7 SAFETY

- 7.1 Follow universal precautions and all required safety procedures when working with potentially hazardous blood, bone marrow, MSCs, etc.

8 PROCEDURE

- 8.1 Quality Control
 - 8.1.1 The Cellometer Auto 2000 does not require any routine testing or calibration. Reference beads can be used for background images if fluorescence detection is needed following repair, movement of the instrument, and/or in troubleshooting unexpected results in the Stem Cell Laboratory.
 - 8.1.2 Daily validation of the Cellometer will be done with beads to ensure the counter is functioning properly; this will also improve the starting point of cell counts are at or close to the optimal z position, or focal plane, which may be disturbed following an interruption in power or system restart.
 - 8.1.3 When performing fluorescence detection and background images, refer to *STCL-EQUIP-022 JA1 Cellometer Auto 2000 Users Manual*.
- 8.2 Daily Validation Bead Check
 - 8.2.1 On days of use, perform a check with validation beads before the first count to ensure optimal starting z position. This only needs to be done once per day on days of use unless further troubleshooting is required.
 - 8.2.2 Load the Assay titled QC Test.
 - 8.2.3 As needed, print a copy of *STCL-EQUIP-022 FRM1 STCL Cellometer Bead Daily Check* for each lot of validation bead solution in stock for

use, and record on it the viability specifications listed on the lot-specific CoA.

- 8.2.4 If the lot has been previously used, confirm the lot matches that recorded on *STCL-EQUIP-022 FRM1 STCL Cellometer Bead Daily Check*.
- 8.2.5 Remove the beads from the refrigerator and invert ten times or vortex for 10 seconds on high.
NOTE: Do NOT shake vial as this will cause air bubbles to form.
- 8.2.6 Pipette 20 µl of bead solution to each chamber of a disposable counting slide.
- 8.2.7 Insert one side of the slide into the Cellometer chamber, name the sample QC-A (for Quality Control slide A), and set the dilution factor to 1.
- 8.2.8 Press “Preview Image for Current Assay”
- 8.2.9 Press button for green fluorescence.
- 8.2.10 Adjust focus so that the green signal is bright with crisp, clearly defined edges.
- 8.2.11 Press “Count Current Image”.
- 8.2.12 Record Viability % on *STCL-EQUIP-022 FRM1*.
- 8.2.13 Press “Begin Next Assay”
- 8.2.14 Name the sample: QC-B (Quality Control slide B), and set the dilution factor to 1.
- 8.2.15 Press button for green fluorescence.
- 8.2.16 Adjust focus so that the green signal is bright with crisp, clearly defined edges.
- 8.2.17 Press “Count Current Image”.
- 8.2.18 Record Viability % on *STCL-EQUIP-022 FRM1*.
- 8.2.19 Average the two viability results, and record the average on *STCL-EQUIP-022 FRM1*. Check to see that the average falls within the specification range listed on *STCL-EQUIP-022 FRM1*/lot-specific CoA.
 - 8.2.19.1 If the average does not fall within range, the failure may be due to pipetting technique, improper mixing, clumping beads, or incorrect settings (wrong assay, incorrect dilution factor).
 - 8.2.19.2 To troubleshoot failing results, first review the counted sample images, and verify all the beads are being counted.
 - 8.2.19.3 If the average fails to meet the lot-specific viability specification, repeat the process outlined above.

- 8.2.19.4 Contact the lab manager or designee if the second sample preparation and counts do not pass.
- 8.2.19.5 Nexcelom may need to be contacted if troubleshooting does not fix the issue.
- 8.3 Starting the Cellometer
 - 8.3.1 Turn on Cellometer Auto 2000 by pressing the power button on the front of the instrument. Cellometer Auto 2000 program will start automatically. DO NOT click on “Cellometer Auto2000” icon on desktop.
- 8.4 Notes on Cell Counts
 - 8.4.1 For optimal performance, it is recommended that the range of total cells counted is between 100 and 5,000 cells.
 - 8.4.2 For 1:2 dilutions with cell counts below 100 total cells counted, contact management.
 - 8.4.3 AOPI should not be added to sample more than 5 minutes before counting. Allowing the cells to sit in AOPI for more than 5 minutes may affect the viability.
- 8.5 Preparation of sample for Dual-Fluorescence Viability using AOPI
 - 8.5.1 The following outlines how to prepare a 1:2 dilution. Do not dilute cells more than 1:2 in AOPI as large amounts of AOPI may be toxic to cells.
 - 8.5.1.1 Remove 20 µl of cell sample and transfer it to an Eppendorf tube. Visually inspect the sample to ensure there are no clumps.
 - 8.5.1.2 Add 20 µl of AOPI staining solution to the Eppendorf tube and gently mix the tube contents. Return AOPI stain to refrigerator when not in use.
 - 8.5.1.3 If a dilution larger than 1:2 is necessary, first dilute the cells in 1X DPBS as outlined in *STCL-EQUIP-022 FRM1*, then prepare a new 1:2 dilution with AOPI.
 - 8.5.1.4 A 1:2 dilution of cells diluted 1:5 with DPBS results in a final 1:10 dilution ($1/2 \times 1/5 = 1/10$). For example: dilute 20 µl of cells in 80 µl of DPBS to create a 1:5 dilution of cell suspension, then in a new tube, mix 20 µl of AOPI with 20 µl of the 1:5 diluted cells in order to create a 1:10 final dilution.
- 8.6 Performing Viability Assay
 - 8.6.1 Select an empty disposable cell counter slide chamber, and if present, remove the protective film from the front and the back of the slide.
 - 8.6.2 Using the stained sample prepared in the steps above, **slowly** transfer 20 µl of sample into the disposable cell counting chamber. Pipette

should be held at a 45° to slide chamber when adding sample to ensure even flow and sample coverage.

NOTE: Careful handling of the disposable cell counting chamber is necessary to prevent leaving prints or residue on the slide. Handle by touching the outside edges of the cell counting chamber during loading and do not tilt chamber vertically.

8.6.3 Allow the chamber to sit for 30 to 40 seconds after loading the chamber; this allows the AOPI to completely penetrate the cells and gives the cells time to settle.

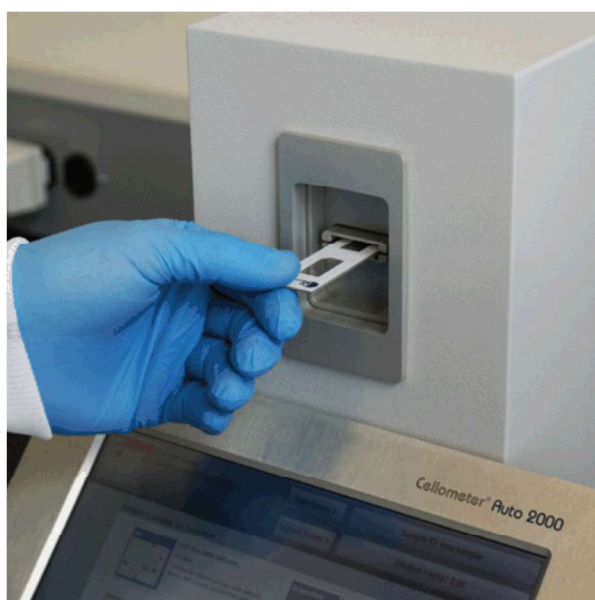
8.6.4 During the 30 second wait, select the appropriate assay for the cell sample using the menu pictured below.

For **freshly harvested MSCs**, use “aGMP fresh MSC” assay. **NOTE:** This program could serve as a backup for the GMP Laboratory should they need to use the STCL’s instrument.

8.6.4.1 For **thawed MSCs**, use “JK Thawed MSC” assay.



8.6.5 Insert the side of the slide containing the cell suspension into the instrument.



8.6.6 Click “Preview Image for Current Assay”. The software will direct the user to create a “Sample ID” for the sample being tested, and then note the “Dilution Factor” used. A bright field image containing cells on the screen will be displayed.

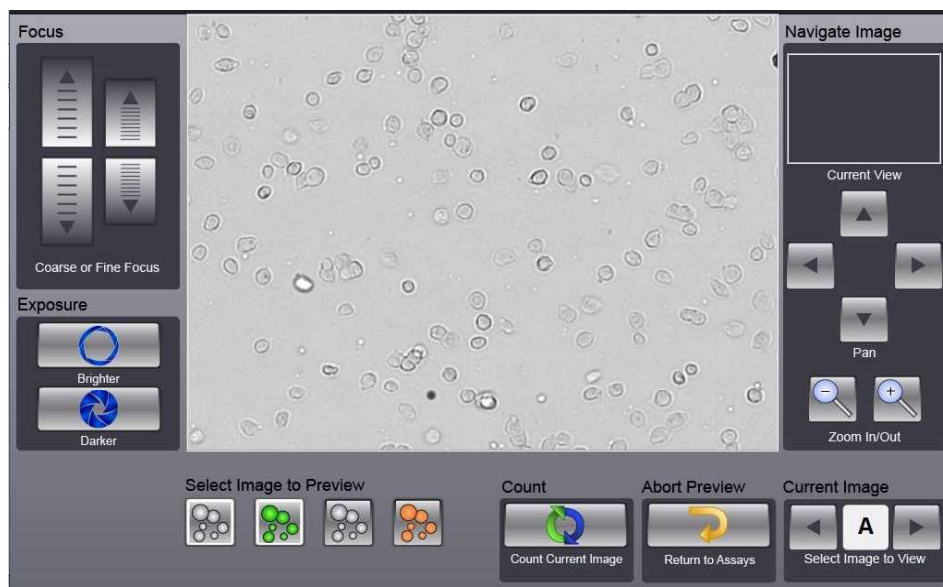
8.6.6.1 If cells were diluted prior to adding AOPI, adjust dilution factor to reflect the final dilution of the cells. For example, enter 10 for a 1:10 dilution.

8.7 Focus Cells

8.7.1 To focus, use the coarse adjustment button (left) and fine adjustment button (right) as shown below. At a minimum, use the fine adjustment to verify that the image has a bright center for each count; the instrument is similar to any microscope, and out of focus or dim cells will not be counted by the program.



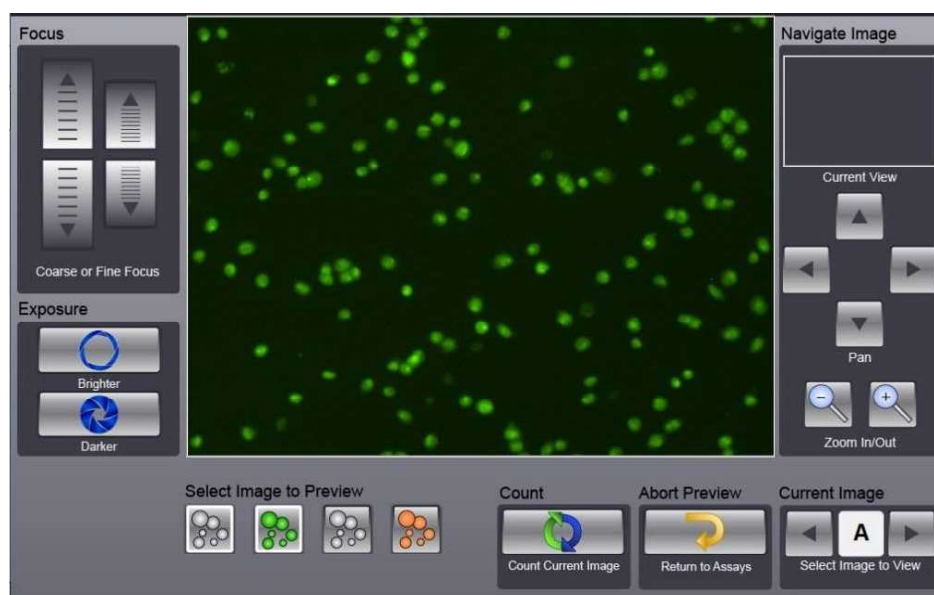
- 8.7.2 Properly focused cells will have a bright center with sharp, crisp edges. The center should be visibly brighter than the background. See the Bright field image below for thawed cord tissue MSCs:



Brightfield Image Preview of Thawed Cord Tissue MSCs

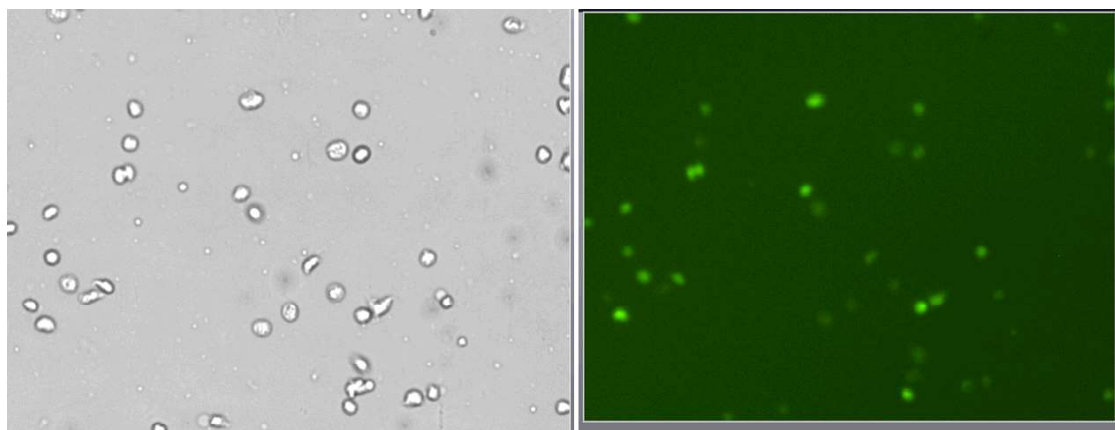
NOTE: The images provided may be followed as a guide; however, the appearance of the cells in bright field may vary once properly focused. To confirm that the images are focused properly, check the green fluorescence as outlined in the next step.

- 8.7.3 Next, preview the green images by clicking on the green icon underneath “Select Image to Preview”, and continue to use the course or fine adjustment buttons to focus the images until the green images are well defined, bright, and not blurry.
- 8.7.4 Focusing the cells in the green image preview is more important than how images appear in bright field.
- 8.7.5 A good example of properly focused green images is shown below for thawed cord tissue MSCs.



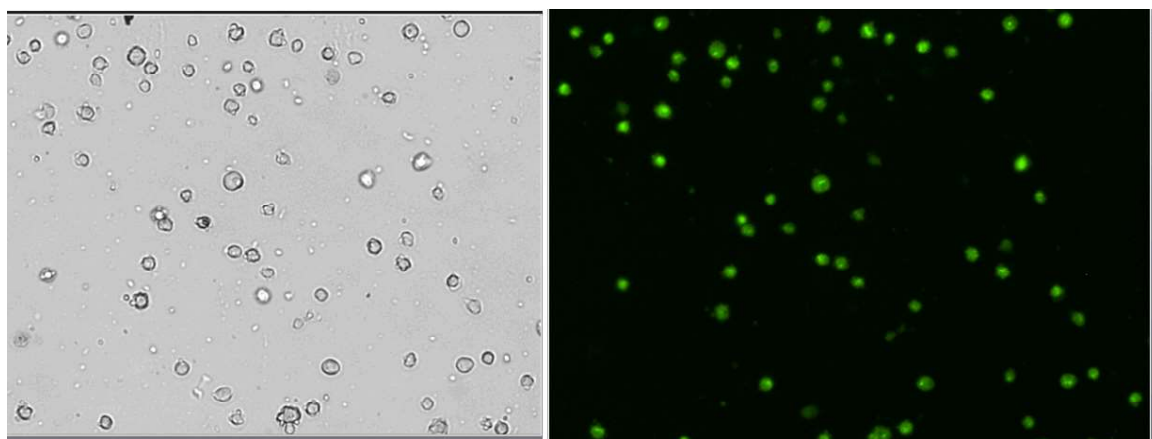
Green Image preview of Thawed Cord Tissue MSCs

- 8.7.6 Additional example images are shown below. The images are provided as a guide, but the appearance of images in bright field may vary slightly when the cells are properly focused.
- 8.7.7 Exposure should only be adjusted when necessary. If the background appears greenish in color, click on Darker. If the green images are dim, check that adjustments to focus won't help before making exposure brighter.
- 8.7.7.1 **NOTE:** Adjusting exposure will change exposure time. Adjustments made to exposure will not reset unless the operator clicks on the home button before counting the next sample. Adjustments to exposure will carry over into the next count if the operator proceeds by clicking on "Begin Next Sample".
- 8.7.8 In the bright field images of thawed cord tissue MSCs below, the edges are not crisp, and in the green images, the cells are not well focused. In the green image preview, the exposure is also too bright since it appears greenish in color.



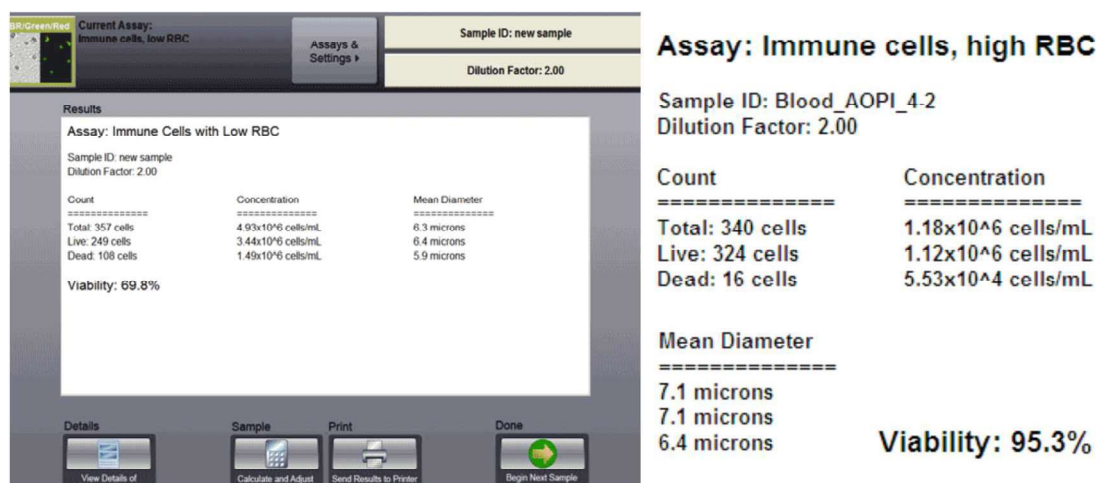
Bright field and Green Image Preview of Thawed cord tissue MSCs

- 8.7.9 In the Bright field image of thawed cord tissue MSCs below, the cell edges are crisp and dark in Bright field. In the preview for green images, the cells appear bright and well defined. The preview of green images confirms that the cells are in focus for counting.



Bright field and Green Image Preview of Thawed cord tissue MSCs

- 8.7.10 Once the image is in focus, press “Count Current Image” to begin the counting process. As the Cellometer Auto 2000 is reading, visually inspect the cells on the screen to verify there are no clumps. If there are clumps, create a new stained cell solution and perform the count again.
- 8.7.11 The results will appear as shown below. To print, press “Send Results to Printer”, and attach the document to the appropriate batch record if necessary.



8.7.11.1 The viability percentage is derived from the following formula:

$$(\text{Total viable cells} / \text{Total cells counted}) \times 100 = \text{Viability \%}$$

8.7.12 The optimal total cell count is between 100 and 5,000 total cells. If documenting the count on *STCL-EQUIP-022 FRM1* for manufacturing and the results are above the optimal total cell count, prepare a higher dilution, and recount the sample. If the results for a 1:2 dilution are below the optimal total cell count, contact the lab manager, medical director, or designee before completing the form.

8.7.13 If performing a second count, carefully turn the cell counting chamber and insert the other side. Press "Begin Next Sample" or click the home button to follow the steps outlined above and repeat the cell count.

8.7.14 After daily processing is finished, the Cellometer Auto 2000 should be shut down by pressing the power button on the front of the instrument.

8.8 Reviewing Cell Count and Transfer form

8.8.1 If reviewing the count on *STCL-EQUIP-022 FRM1* for manufacturing, confirm that the dilution factor reported on the form matches the dilution factor listed on the result page printed from the Cellometer and that the correct assay was used.

8.8.2 During the review of a count for manufacturing purposes, check for any additional errors before proceeding.

8.9 Modifying Assays

8.9.1 When creating new assays from an existing assay, the user must select the assay of interest, click on edit, rename the assay, and if applicable, select to unlock the new copy before clicking on save.

8.9.2 Creating an assay requires selecting, reusing, or modifying a Cell Type which contains most of the counting settings.

8.9.3 When creating a new assay from an existing assay, it is advised to select "Change" when prompted to keep or change the Cell Type. This will ensure any changes made to the Cell Type won't affect other assays.

8.9.4 Once locked, changes to an assay can't be made; however, if the Cell Type within the locked assay is unlocked, any changes made to the unlocked Cell Type will carry over into any locked assays that share the same Cell Type. For this reason, it is recommended to lock both assay and Cell Type.

8.9.5 To check on the current settings for "JK Thawed MSC," refer to *STCL-EQUIP-022 JA2 Cellometer Program Used for Thawed MSC Products*.

8.9.5.1 For reference, the starting Cell Type used to develop the most recent assays used to count thawed MSC's was Assay 2 Immune Cells Live for Channel 1 and Assay 2 Immune Cells Dead for Channel 2, but settings to this Cell Type were modified and renamed as listed on *STCL-EQUIP-022 JA2*.

8.9.6 Once a Cell Type is locked, it may be difficult to view the settings; however, some of the settings may be printed by selecting the appropriate assay, clicking on "edit", and then click on "print" at the top of the Assay Parameters window.

8.10 Maintenance

8.10.1 Preventive maintenance is performed annually by Nexcelom.

8.10.2 On rare occasions, dust may build up in the chamber. This may appear as a "black cloud" on the Cellometer screen. The light pathway of the device needs to be cleaned. Use a computer duster can of compressed air to blow a quick burst of air into the unloaded chamber. Use short bursts of air rather than extended bursts to clear the dust. The can should be held upright to no moisture enters the chamber. Perform a run on the Cellometer with an empty chamber to confirm the image is clear. If this does not resolve the issue, contact Nexcelom for further assistance.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-EQUIP-022 JA1 Cellometer Auto 2000 Users Manual

9.2 STCL-EQUIP-022 JA2 Cellometer Program Used for Thawed MSC Products

9.3 STCL-EQUIP-022 FRM1 STCL Cellometer Bead Daily Check

9.4 STCL-FORM-074 Processing Lot Numbers – MSC Thaws

10 REFERENCES

10.1 Nexcelom Cellometer Auto 2000 User Manual 2015.

11 REVISION HISTORY

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