SOP Title: Use and Maintenance of the Cellometer Cell Counter		
Document ID: 26004	Version	4.0
Page 1 of 15	Supersedes	3.0
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1. PURPOSE

1.1 The purpose of this procedure is to describe the use and maintenance of the Nexcelom Cellometer Auto 2000.

2. SCOPE

2.1 This procedure applies to all cell counters.

3. REFERENCES

3.1 Cellometer Auto 2000 User Manual

3.2 10007: Non-Routine Equipment Maintenance

3.3 10009: General Record Review

3.4 15000: Waste Disposal at the Advanced Technology Research Facility (SOP)

4. RESPONSIBILITIES

4.1 The Research Associate, hereafter referred to as Analyst, is responsible for reviewing and following this procedure, and documenting performance of equipment maintenance.

4.2 The Quality Control Analyst is responsible for reviewing and following this procedure. Quality Control Analyst is responsible for maintaining monthly instrument verifications.

4.3 The Scientific Manager or designee is responsible for training personnel in this procedure and reviewing associated documentation.

4.4 The Quality Assurance Specialist is responsible for quality oversight and approval of this procedure.

4.5 Trained personnel perform equipment maintenance record review per “10009: General Record Review.”

5. DEFINTIONS

5.1 As Needed Maintenance – maintenance that is performed outside of routine maintenance but is not performed in response to equipment malfunction.

5.2 Routine Maintenance – maintenance that is performed at planned intervals to identify and prevent problems before they result in equipment failure.

5.3 Non-Routine Maintenance – maintenance that is performed in response to equipment malfunction or failure.

6. REAGENTS, MATERIALS, AND EQUIPMENT

6.1 Nexcelom Cellometer

6.2 Cellometer Check Validation Bead Solution (Nexcelom, Cat # CCBM-011-2mL)

6.3 Class II Biosafety Cabinet (BSC)

6.4 Hemocytometer, Disposable (Nexcelom, Cat # CP2-002 or equivalent)

6.5 Pipettes

6.6 Pipette Tips

6.7 Primary Disinfectant (Cavicide, Warehouse, Cat # 79300360 or equivalent)

6.8 Secondary Disinfectant (Ster-ahol, VWR, Cat # 14003-358 or equivalent)

6.9 ViaStain AOPI Staining Solution (Nexcelom, Cat # CS1-0106-5mL)

6.10 Wipe, Low-Lint, Wypalls (Warehouse, Cat # 79300335 or equivalent)

7. HEALTH AND SAFETY CONSIDERAIONS

7.1 Proper safety precautions should be taken while working in a laboratory setting. This includes, but is not limited to, proper protective equipment such as lab coats, safety glasses, closed-toe shoes, and non-latex gloves.

7.2 Refer to the respective Safety Data Sheet (SDS) when working with any chemicals.

7.3 Refer to “15000: Waste Disposal at the Advanced Technology Research Facility,” “EHS-WM-1: Disposal and Minimization of Chemical Waste,” and “EHS-WM-2: Biological Waste Handling and Disposal” for waste disposal processes.

8. START UP

8.1 Turn on Cellometer by pressing power button on front of unit.

8.2 Click Nexcelom icon

8.3 Verify Cellometer is connected to network.

8.3.1. Click Windows Start icon.

8.3.2. Select “computer” and double click on the correct network drive to verify it is connected without any errors. Login to network drive using your NIH credentials.

8.4 Prior to use, confirm the Quality Check was performed for the month prior per section 10. If it has not been performed, perform Quality Check per section 10.1, and make entry on “26004-01: Cellometer Monthly Maintenance Form.”

9. COUNTING CELLS

9.1 Inside BSC, mix cell pellet with cell culture media until mixture is homogenous.

Note: A cell concentration range of 1.0 x 10^5 to 1.0 x 10^7 cells/mL can be analyzed by the Cellometer. A concentration of 1.0 x 10^6 is optimal. If undiluted cells are expected to be at a greater concentration than 1.0 x 10^6, dilute cells in media according to Table 1, as needed.

Table 1: Pre-Dilution Values (As needed)

Dilution Factor	Volume of Cell Mixture (µl)	Volume of Media (µl)	Total Volume (µl)	Final Dilution Factor (after adding AOPI Stain)
2	50	50	100	**4**
3	30	60	90	**6**
4	25	75	100	**8**
5	20	80	100	**10**

9.2 Add 20 µL of cell mixture to two microfuge tubes.

9.3 Add 20 µL of AOPI Stain to tube 1. Pipette suspension at least 5 times up and down to mix.

9.4 Add 20 µL of AOPI Stain to tube 2. Pipette suspension at least 5 times up and down to mix.

9.5 Using a pipette, mix tube 1 at least 3 times up and down then immediately add 20 µL of cell/AOPI Stain solution to side 1 of the hemocytometer chamber.

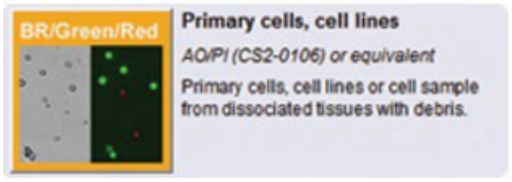
Note: If preferred, label slide chamber #1 and chamber #2 on white margin of chamber. Avoid touching clear portion of counting chamber.

9.6 Insert loaded chamber into Cellometer sample slot and gently push slide until it stops.

9.7 Ensure appropriate final dilution factor is listed on Cellometer home screen (Image 1.) Without further dilution as is noted in Table 1, the dilution factor will be listed as “2” (20 µL cells + 20 µL AOPI Stain).

Image 1: Cellometer Home Screen

9.8 Select the “Primary cells, cell lines” icon on the home screen.



9.9 Select “Preview Image for Current Assay” icon.

9.10 Enter analyst initials in “Enter User ID” in top left of screen using either touch pad or keyboard.

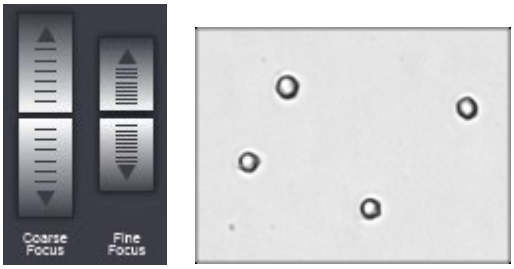
Note: Use the following format to “Enter Sample ID”:

Logbook Number / Lot Number_Date_Count #

(e.g., P0001_01Mar19_1)

9.11 Select “Save” at bottom right hand side of screen.

9.12 Adjust focus, if necessary, using coarse and fine adjustments on left-hand side of screen. Cells in focus have a bright center and a crisp edge.

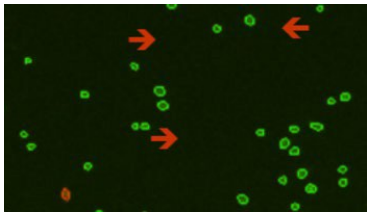


9.13 Select “Count” icon at bottom of screen.

9.14 Select “View Details” icon at bottom left of screen, then select “View Counted Image” icon on left-hand side of screen.

9.15 Review counted image. Nucleated cells with intact membranes stain fluorescent green and are counted as live, whereas nucleated cells with compromised membranes only stain fluorescent red and are counted as dead.

Note: Non-nucleated material, such as red blood cells, platelets and debris, do not fluoresce and are not counted by Cellometer software.



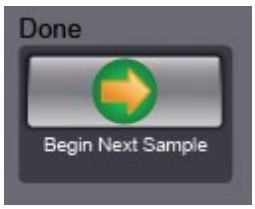
9.16 Select “Return” or “Back to Results” icon. Cell count, concentration, mean cell diameter, and % viability are displayed. Record concentration and % viability on associated form or in a Laboratory Notebook.

Note: Ensure “live cell count” concentration is recorded for cell concentration (number of cells).

9.17 The Cellometer is now ready to analyze next sample.

9.18 Using a pipette, mix tube 2 at least 3 times up and down then immediately add 20 µL of cell/AOPI Stain solution to side 2 of hemocytometer chamber.

9.19 Insert imaging chamber loaded with sample 2; select “Next Sample” icon at bottom right of monitor screen.



9.20 Enter Sample ID when prompted as described in step 9.11, then click “Count”.

9.21 Remove slide from counting chamber after use and discard.

9.22 Select “Return” or “Back to Results” icon. Cell count, concentration, mean cell diameter, and % viability are displayed. Record results per step 9.17.

9.23 Turn instrument off after use and clean up any spills that may have occurred with Primary Disinfectant.

10. MAINTENANCE

10.1 Monthly Maintenance

10.1.1. Cellometer Quality Check

Note: The Quality Check is performed each month that instrument is in use, PRIOR to any cell counts being performed.

Note: The Quality Check is performed by Quality Control Analyst or designee.

10.1.2. Obtain the Certificate of Analysis (CoA) for the Reference Bead Solution. Record Manufacturer Lot Number, Quality Control Concentration (Bead Solution Range), and Viability Specification (% Green FL Beads) ranges on “26004-02: Cellometer Monthly Quality Check Form.”

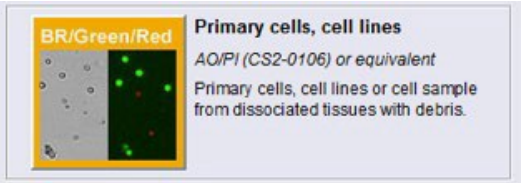
10.1.3. Obtain a hemocytometer chamber and remove plastic covering from both sides.

10.1.4. Vortex Reference Bead Solution for ten seconds at max level prior to use.

10.1.5. Invert Reference Bead Solution ten times and immediately load 20 µL into 1 sample slot of the hemocytometer chamber.

10.1.6. Gently insert loaded chamber into Cellometer and push until it stops.

10.1.7. Select Primary cell, cell lines, AO/PI icon on instrument display.



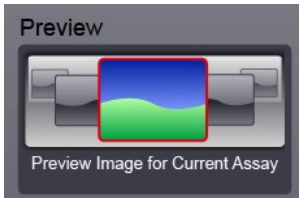
10.1.8. At top right of display screen, select “Sample ID” icon.

10.1.9. Use either the touch pad or keyboard to enter the following format: Ref Bead_Date_Analyst Initials_Count # (e.g., Ref Bead_01Mar19_ABC_1)

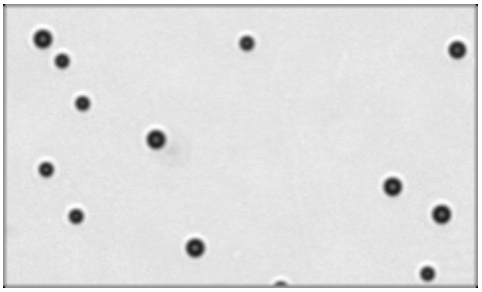
10.1.10. Select “Save” at bottom right hand side of display screen.

10.1.11. Enter a Dilution Factor of 1.00.

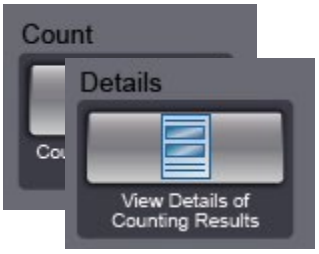
10.1.12. Select “Preview Image for Current Assay” icon on display screen.



10.1.13. Adjust focus, if necessary, by using coarse and fine adjustments on left hand side of screen until the best bead counting focus is achieved. The beads appear as dark circles with sharp edges.

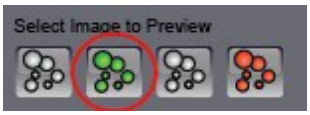


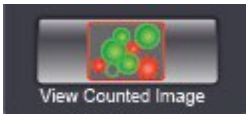
10.1.14. Select “Count” icon at bottom of display screen.

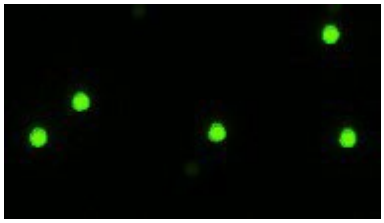


10.1.15. When counting is complete, select “View Details” icon at bottom left of display screen.

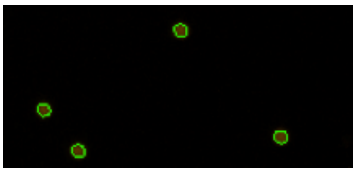
10.1.16. Select the green fluorescent image, then click on “View Details of Counting Results” icon on bottom, left-hand side of screen. Enlarge image by clicking “Zoom In” icon on right-hand side of screen. Confirm all of the green fluorescent beads are circled in green.







10.1.17. Select the red fluorescent image. Confirm all red fluorescent beads are circled in green.



10.1.18. Imaging and counting in bright field, green fluorescent, and red fluorescent channels are now confirmed.

10.1.19. Record “Total” results under “Measured Concentration (Cells/mL)” and the Viability under “Measured Viability (%)” on 26004-02. Remove slide from counting chamber and discard in a pipet tip box.

10.1.20. A passing Quality Check produces results that are within manufacturer’s Quality Assurance range described on the CoA.

10.1.21. If Quality Check fails to be within manufacturer’s range, repeat steps 10.1.3 to 10.18.

10.1.22. If Quality Check fails to be within expected range a second time, obtain a new vial of Reference Bead Solution, new 26004-02 form and repeat steps 10.1.2 to 10.1.18.

10.1.23. If Quality Check fails a third time, immediately stop using Cellometer and contact Scientific Manager or designee for next steps.

10.1.24. Turn Cellometer off after use.

10.1.25. Record maintenance on 26004-01.

10.2 As Needed Maintenance

10.2.1. Spills

Note: Clean up all spills immediately.

Note: Ensure drawer slide is closed and Plate Reader is turned off before cleaning.

10.2.2. Spray Cavicide on a low-lint wipe and wipe the outside surface of the machine. DO NOT spray directly onto Cellometer.

10.2.3. Document As Needed Maintenance in its respective section on 26004-01.

10.3 Non-Routine Maintenance

10.3.1. In the case that the Cellometer is not operating correctly, transition processes being performed to another unit (when applicable), post a sign stating the equipment is out of service and initiate non-routine maintenance documentation per “10007: Non-Routine Equipment Maintenance.”

10.3.2. Document the nature of any failures or malfunctions, how and when it was discovered, and the personnel involved on “10007-01: Non-Routine Equipment Maintenance Form.”

10.3.3. Initiate a service request and complete the non-routine maintenance process following 10007.

11. ATTACHMENTS

11.1 Attachment 1: 26004-01: Cellometer Monthly Maintenance Form

11.2 Attachment 2: 26004-02: Cellometer Monthly Quality Check Form

12. REVISION HISTORY

Version	Change	Reason
1.0	Create new SOP for the use and maintenance of the Cellometer Auto 2000	New instrument SOP.
2.0	Update weekly Ref Bead Check to monthly. Include that dilution factor set to 1. Add details to Section 10.	Counts are consistent and it is wasting reagents. Provide easier instructions to follow and distinguish between sample and ref bead check.

Version	Change	Reason
3.0	1. Updated procedure to new format; forms now separate. 2. Minor formatting and grammar revisions throughout procedure. 3. Added user manual and removed HSL_EQ_003, HSL_EQ_007, HSL_GL_002, HSL_GL_003, HSL_GL_006, HSL_GL_007, HSL_GL_008, HSL_GL_009 and HSL_GL_010 from References section. 4. Added BSC, cellometer, pipettes and pipette tips to materials section. 5. Removed ATRF, HSL, HPV, SOP and BSC from Definitions section. 6. Revised HSL_EQ_006.01 to record monthly maintenance performance. 7. Created form HSL_EQ_006.02 to record monthly bead check information.	1. Consistency between procedures; ease of use. 2. Clarification. 3. User manual used to write procedure; procedures not referenced in body of procedure. 4. Materials used in procedure. 5. Definitions either recorded in earlier section of procedure or not referenced in procedure. 6. Streamlining tracking of maintenance; use tracked on process related documents. 7. Ease of use.
4.0	1. Add Non-routine maintenance. 2. Clarified quarterly maintenance 3. Minor formatting and grammar revisions throughout procedure. 4. Updated Protocol nomenclature	1. New SOP standardization process 2. Ease of use 3. Clarification 4. GDP compliance

Attachment 1: 26004-01: Cellometer Monthly Maintenance Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: Cellometer Monthly Maintenance Form			
Document ID: 26004-01		Version:	4.0
Associated SOP: 26004		Effective Date:	13Aug21
Supersedes Version:	3.0	**Page 1 of 1**	

Equipment ID:		Maintenance Year: (YYYY)	

Monthly Maintenance (See: 26004-02: Cellometer Monthly Quality Check Form)

Month	January	February	March	April	May	June
Recorded by/date:						
Reviewed by/date:						
Month	July	August	September	October	November	December
Recorded by/date:						
Reviewed by/date:						

As Needed Maintenance ☐ N/A

Date	Activity Performed	Recorded by/date	Reviewed by/date
☐ N/A			
☐ N/A			

QA Reviewed by/date: _____

Verify current version prior to use. Use of a superseded or obsolete document is prohibited.

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Attachment 2: 26004-02: Cellometer Monthly Quality Check Form

Frederick National Laboratory for Cancer Research sponsored by the National Cancer Institute		Vaccine, Immunity and Cancer Directorate Standard Operating Procedure Form	
Form Title: Cellometer Monthly Quality Check Form			
Document ID: 26004-02		Version:	4.0
Associated SOP: 26004		Effective Date:	13Aug21
Supersedes:	3.0	**Page 1 of 1**	

Equipment

Description	Identification Number
Cellometer Auto 2000	

Reagents

Description	Lot Number	Expiration Date
Reference Bead Solution		

Results

Reference Bead Solution Range (Beads/mL)	Measured Concentration Range (Cells/mL)	Result
		☐ Pass ☐ Fail
Reference Bead Solution Viability Specification (%)	**Measured Viability (%)**	**Result**
		☐ Pass ☐ Fail

Comments:
☐ First fail, repeat.
☐ Second fail, obtain new vial of Reference Bead Solution and repeat.
☐ Third fail, equipment placed out of service and Scientific Manager contacted.

☐ N/A

Performed by/date:	
Reviewed by/date:	
QA Reviewed by/date:	

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