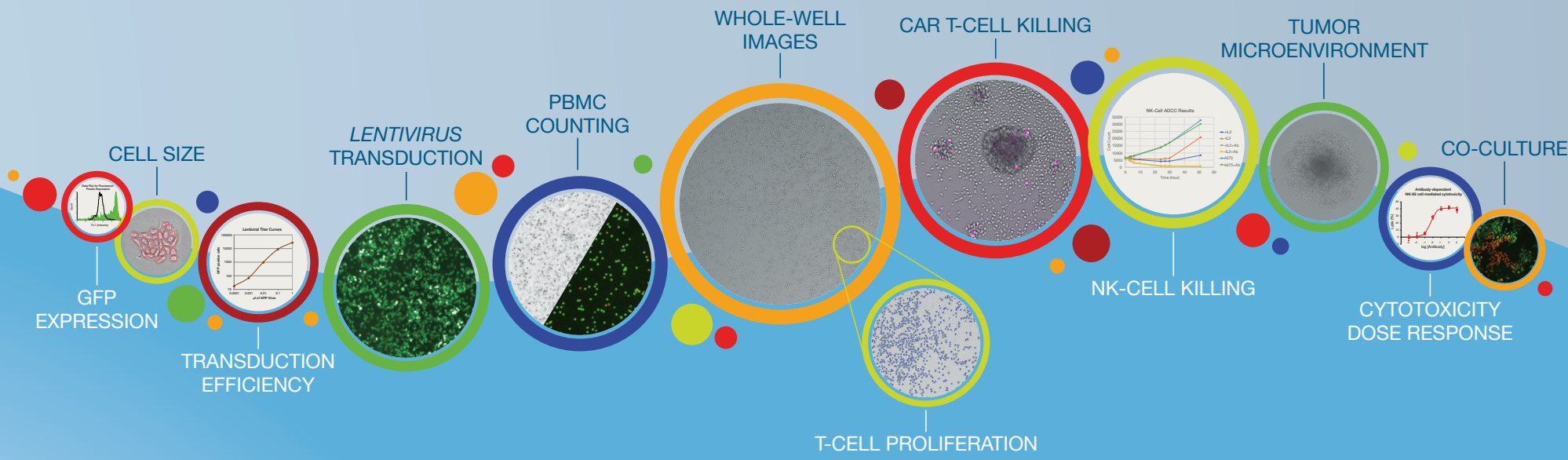
Accurate Cell Counters for CAR T Therapy

from Discovery to Manufacturing



Nexcelom Bioscience

Accurate Cell Counting Methods, Fit for Purpose, for CAR T Therapy

1. **Fast Accurate Counting for Primary Samples**

2. **Overcoming morphology variability in cancer cell lines**

3. **Only one cell counter is needed throughout the (CAR) Chimeric Antigen Receptor T-Cell Development**
   Improve consistency, accuracy and efficiency

4. **Optimize Viral Vector Development with Image Cytometry**
   Improved transfection, transduction and viability

5. **Accurate cell counting assay of apheresis materials - suitable for stability program**

6. **Cell Counting Method Development Process**

"Our Cellometer Auto 2000 has greatly improved the speed and accuracy of our cell counting and has allowed us to analyze our cell samples in a variety of ways. The AOPI feature has been integral in generating cell viable data for a few of our new projects. The customer service team is also amazing! They are very knowledgeable about their products and technology and are always available to help us troubleshoot." - NIH-NIDCD

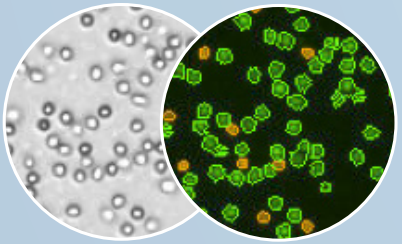
"We have been using the Cellometer Auto 2000 to count splenocyte samples without RBC lysis. The Cellometer Auto 2000 and the AOPI Viastain gives us the ability to get an accurate count of our live splenocytes while excluding the red blood cells." - Eli Lilly

"…definitely the standard for cell counting. Results are consistent time and again, the reliability of which is great when performing duplicate or triplicate counts…. I also enjoy the ability to establish several different settings based on the type of cell, and also appreciate Nexcelom's customer service!" - Cold Spring Harbor Laboratory

Simple Cellometer Workflow

1. Pipette 20 µl

2. Insert slide & count



3. Get images & data

Accurate Cell Counters for CAR T Therapy

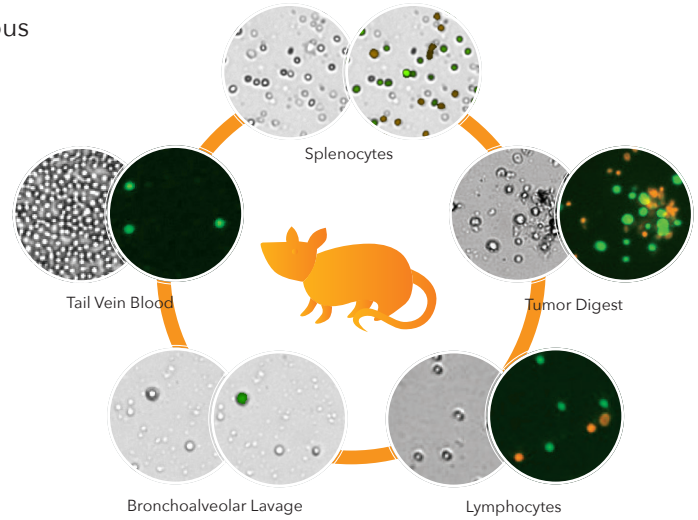
1 Fast Accurate Counting for Primary Samples

Mouse samples are variable in cell type and phenotypic characteristics.

- Cell counting accuracy can be affected by heterogeneous cell populations
- Nuclear staining is critical for accurate cell counting

"The Cellometer Auto 2000 has greatly aided the development of our tissue dissociation assays. The unit allows fast quantification of viable white blood cells from a heterogeneous population following spleen, thymus, lymph node, etc. dissolution which can then quickly proceed to downstream flow-based analysis. The time saved by this method has been extremely valuable!" - Takeda Pharmaceuticals

✓ Cellometer provides accurate concentration and viability for cells that vary in morphology and heterogeneity.



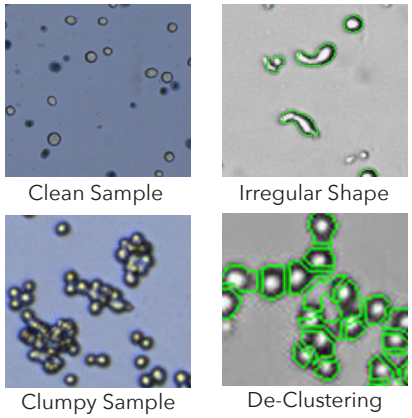
Ezeh PC, et al. *PLoS One*. 2014; 9(4):e93920 | Xu H, et al. *Toxicol Lett*. 2016; 262:55-61

2 Overcoming Morphology Variability in Cancer Cell Lines

Advanced imaging and analysis algorithms are necessary to address the cell morphology diversity in the large tumor cell lines. It is important for the cell counter to measure cells with these characteristics accurately.

"We use the Cellometer Auto 2000 daily to count cells and assess viability of cells derived from a variety of sources. [We] perform a lot of cell line work, and need accurate counts and viability assessments before using the cells in downstream assays" - Regeneron Pharmaceuticals

✓ Cell counters need to handle NCI-60 cell lines, of which 57% are clumpy, clustering, contain debris or have large variations in morphology.



Rothenberg SM, et al. *Cancer Res*. 2010; 70(6): 2158-64

3 Only One Cell Counter is Needed Throughout CAR T Development

Cell counters and image cytometers are critical throughout the CAR T process

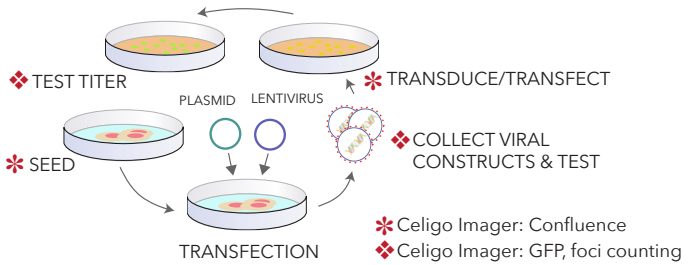
- Accurate cell counting from Leukopaks
- Measuring transduction efficiencies
- Determining efficacy of engineered CAR T-cells

Singh H, et al. *PLoS One*. 2013; 8(5): e64138 | Wang X, et al. *Cancer Gene Ther* 2015; 22(2):85-94

4 Optimize Viral Vector Development with Image Cytometer

Accurate cell number and viability measurement is necessary for viral vector development and downstream manufacturing processes.

- Assure correct plating density, cell confluence
- Determine transduction efficiencies (No trypsinization required)
- Verify cell number and viability for plating



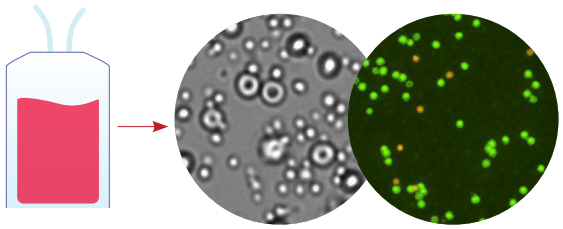
"The Cellometer Auto 2000 has made functional testing of T cells a much quicker and simpler process than before! When the cell concentration of each population in a 96 well plate must be determined, it is hard to imagine having to count the cells by hand and doing this in a timely manner. Great product!"
- University of Texas at Austin

White KM, et al. ACS Infectious Diseases 2018; 4(2):146-157 | Zhang Z, et al. BMC Biotechnology 2018; 18(1):4

5 Accurate Cell Counting Assay of Apheresis Materials - Suitable for Stability Program

Counting assay, of difficult to measure apheresis material, for stability programs including shipping, handling and storage conditions.

- Measure viable and dead nucleated cells
- No RBC lysis necessary
- No staining-incubation period



"…We routinely process PBMCs from both fresh whole blood and from frozen stock. The Cellometer [Auto2000] has made it much easier to get cell numbers and viability percentages for use in downstream applications …"
- Human Longevity, Inc

✓ Manual / trypan blue method is not accurate for counting leukapheresis. RBCs are 3-12x the number of lymphocytes.

✓ Proven Repeatability: 14 PMBC samples were tested using the Cellometer Auto2000. The resulting in a CV of < 6%.

Burchiel SW, et al. *Inhal Toxicol*. 2016; 28(2):61-70

6 Cell Counting Method Development Process

Counting is Crucial During All Stages of Cell-based Product Development

◆ Cellometer Cell Counting
✤ Celigo Imaging Cytometer

✓ ≥70% cell viability is recommended for pre-clinical safety studies and clinical dose.

✓ Cellometer K2 can measure cell viability from 0% viable to 100% viable cell population.

6 Cell Counting Method Development Process *(continued)*

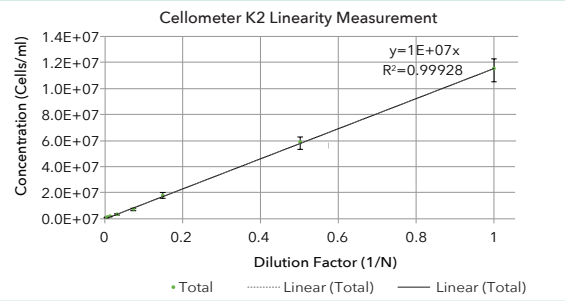
Establishing Early and Robust Cell Counting Procedures During Cell-Based Product Development is Crucial for Many of These Parameters

PRE-CLINICAL
- Selection
- Development
- Optimization

PHASE I
- Methods qualified
- Set tentative release/ stability criteria

PHASE II
- Assay optimization
- Consider validation acceptance criteria
- Delineate/initiate assay validation parameters

PHASE III
- Full assay validation by licenser (recommended by Phase III)
- Biologics license applications



Cell Type: Jurkat Staining Method: AO/PI N=10 Range: 1x10^5 to 1x10^7

- Linearity
- Accuracy
- Range
- Intermediate precision
- Precision: repeatability

and more . . .

Intermediate Precision: Instrument Repeatability and Validation

Cellometer Auto 2000	Average Total Concentration	Average % Viability
Instr. # 1	1.98E+06	75%
Instr. # 2	2.00E+06	74%
Instr. # 3	1.98E+06	74%
Instr. # 4	1.97E+06	74%
Instr. # 5	2.01E+06	74%
Instr. # 6	1.98E+06	74%
Instr. # 7	1.99E+06	74%
Instr. # 8	1.98E+06	74%
Average	1.99E+06	7.42E-01
STDEV	1.22E+04	1.71E-03
%CV	0.61%	0.23%

8 Different Cellomter Auto 2000s were tested
For each Cellometer, 10 individual samples counts were performed

Precision: Repeatability of Cellometer K2

	Live Cell [ ]	Percent Viability
Mean	5.94E+06	96.0%
CV	4.69%	0.54%

Cell Type: PBMC Staining Method: AO/PI N=10 Range: 1x10^5 to 1x10^7

"I am currently using the Cellometer K2 in our lab, mostly to count T cells, PBMCs, and tumor cells. I use them for cell culture, and later the cells are used for further assays like ELISA and FACS. The instrument is very accurate, especially with AO/PI."
- EMD Serono

✓ Accuracy translates from cell lines to primary samples.

✓ Cellometer cell counters have been facilitating CAR T manufacturing with accurate cell counting and analysis since 2010.

Jin J, et al. *J of Transl Med* 2018; 16(1):13 | Singh H, et al. Cancer Gene Ther. (2015) 22(2):95-100 | Manuri, PVR, et al. Human Gene Therapy. 2010; 21:427-437

Which Instrument is Right for Me?					
Features	**Fluorescent Viability Cell Counters**		**Image Cytometers**		
	Auto 2000	**K2**	**Celigo BF**	**Celigo 4 Channel**	**Celigo 5 Channel**
Cell / Sample Type					
Cell Line	X	X	X	X	X
Cultured Primary Cells	X	X	X	X	X
Low Concentration Cell Lines	X	X	X	X	X
Primary cells (Messy Sample*)	X	X		X	X
PBMCs, Splenocytes, Stem Cells	X	X		X	X
Hepatocytes		X		X	X
Adipocytes***	X	X	X	X	X
Cell-Based Assay **		X	X	X	X
Apoptosis (Annexin V-FITC/PI)		X		X	X
Apoptosis (Caspase Activity)		X		X	X
Cell Proliferation (CFSE)				X	X
Cell Cycle (PI)		X		X	X
GFP Transfection	X	X		X	X
RFP Transfection				X	X
Mitochondrial Potential (JC-1)				X	X
Multi-drug Resistance (ABC Transporter)				X	X
Surface Marker Analysis				X	X
Vitality (Calcein-AM/PI)		X		X	X
Image Cytometry**				X	X

**A messy sample is a heterogeneous sample containing unwanted cell types, such as red blood cells, in addition to the cells of interest.*
*** FCS Express license must be purchased in order to perform Cell Based Assay or Image Cytometry analysis*
**** Cellometer CHT4-PD300 slides are required for cells greater than 80µm in diameter*

Innovation and Expertise in the Science of Cell Counting

Schedule a FREE on-line demonstration, on-site demonstration or technical seminar with a Nexcelom Applications Specialist today.

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