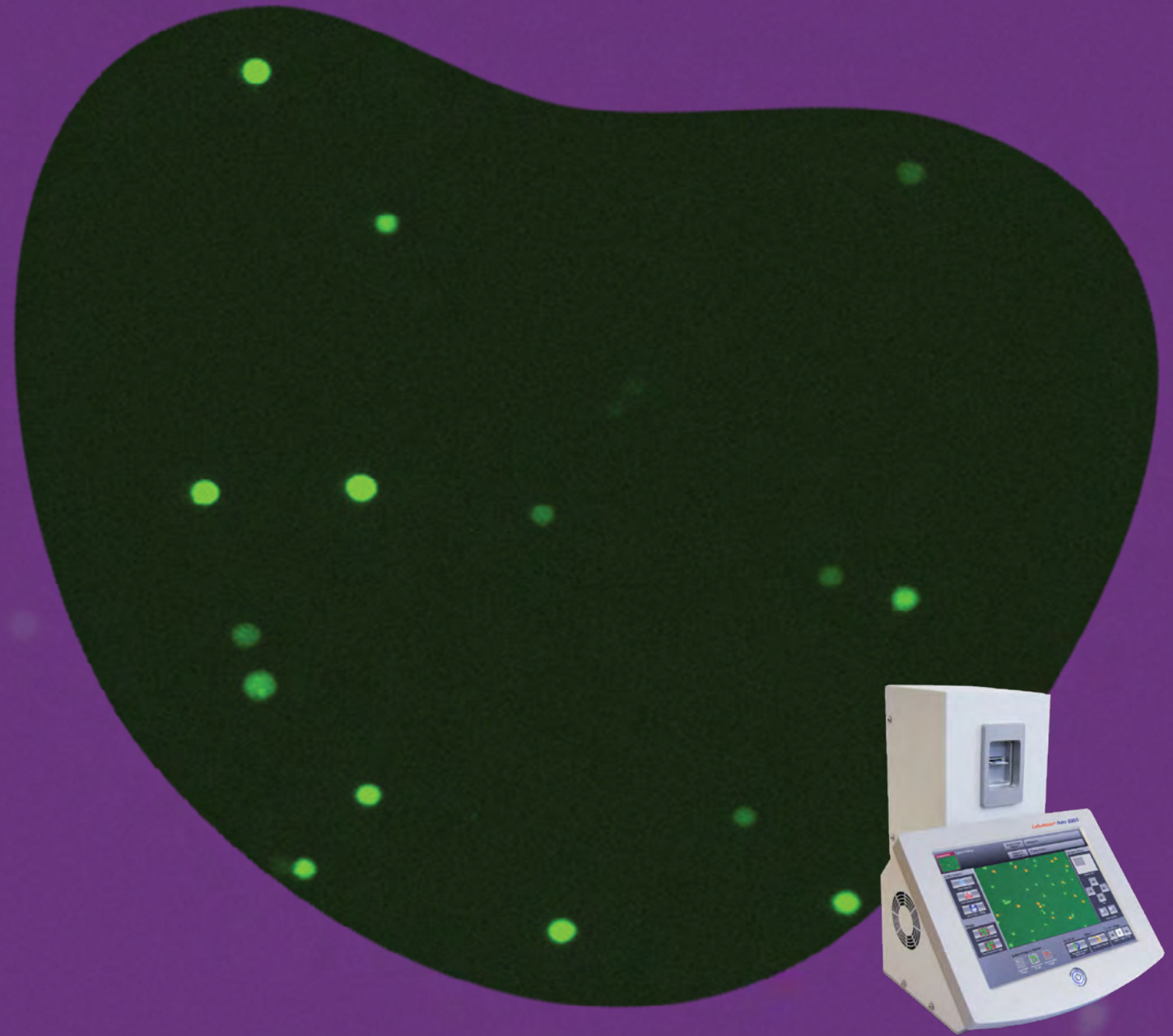


Cell viability
counter for
primary cell
analysis.

revvity



Cellometer™ Auto 2000 cell viability counter

Optimized analysis of primary cells

Features of the Cellometer Auto 2000

Dual fluorescence and brightfield imaging: Staining of both live and dead cells in heterogeneous samples

Integrated system: Simple, space-saving design

User-friendly touchscreen and assay selection: Enhanced inter-operator reproducibility, minimal training, auto-save option

Fast results: Obtain cell images, counts, size measurements, and viability calculations in 30 seconds

Small sample size: Only 20 μl of sample

Broad dynamic range: Measurable concentration range of 1×10^5 to 1×10^7 cells/mL using Revvity's patent-pending de-clustering function

Many compatible dyes: Trypan blue, AO, PI, EB, 7AAD, AO/PI, AO/EB, Calcein AM, CFDA, Calcein AM/PI, CFDA/PI



| Cellometer Auto 2000 cell viability counter



Imagery for greater results

Advantages of Cellometer Auto 2000 cell viability counter

Cell imaging

- Verify cell morphology and counted live/dead cells
- Export cell images for presentations and publications

Pattern recognition software

- Accurately count cells in clumps
- Count irregular-shaped cells
- Eliminate debris from cell counts
- Differentiate cells based on size

Automated data management

- Pre-set assays and automated reports
- Archive sample images and auto-save results

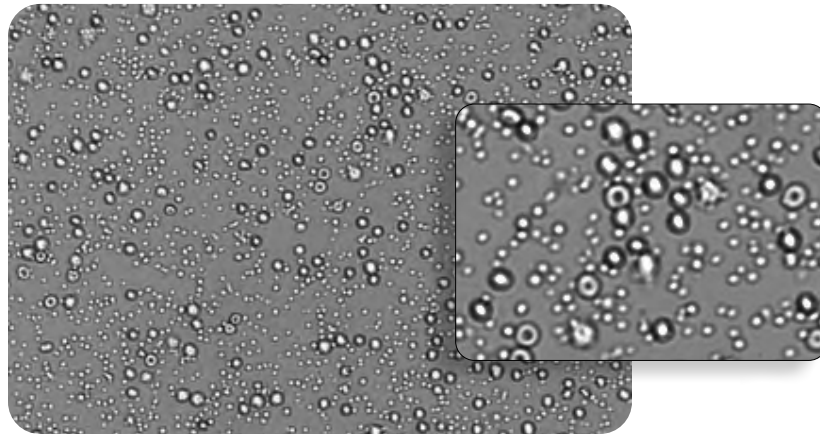
Maintenance-free system

- Disposable counting chambers – no wash steps
- No required instrument maintenance

“I like the Cellometer Auto 2000 because it eliminates manual counting and our counts are consistent between users. We count cells from primary samples and I like that the RBCs are not counted when we use the AO/PI stain”

- Moffitt Cancer Center

Primary cell analysis



PBMC analysis in the presence of red blood cells

Measure PBMCs from whole blood without lysing. Obtain baseline PBMC concentration and viability prior to biomarker studies.

Nucleated cell concentration and viability

Evaluate cord blood and bone marrow samples

GFP transfection efficiency and viability

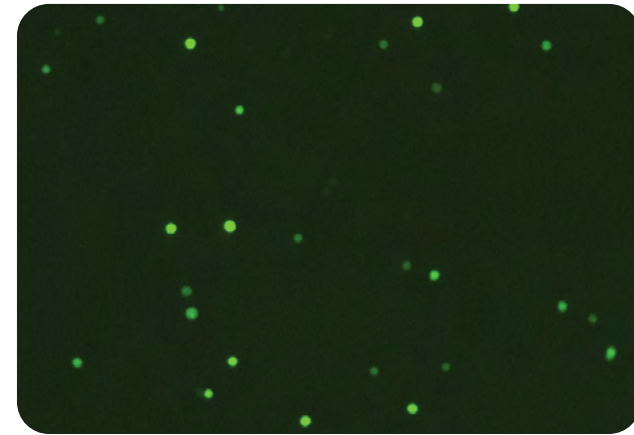
Quickly and easily monitor DNA, RNA, and siRNA transfection

Analysis of clumpy and irregular-shaped cells

Revvity's exclusive pattern-recognition software enables accurate analysis of >98% of mammalian cell types

Cell line analysis

Automatically capture fluorescent cell images, concentration, Trypan blue or PI viability, and mean diameter in 30 seconds!



Primary cell analysis

Accurate concentration and % viability for primary cells (PBMCs, stem cells, splenocytes, neural cells, and more)

Analysis of cells from heterogeneous samples

- Whole Blood
- Peripheral Blood
- Cord Blood
- Bone Marrow

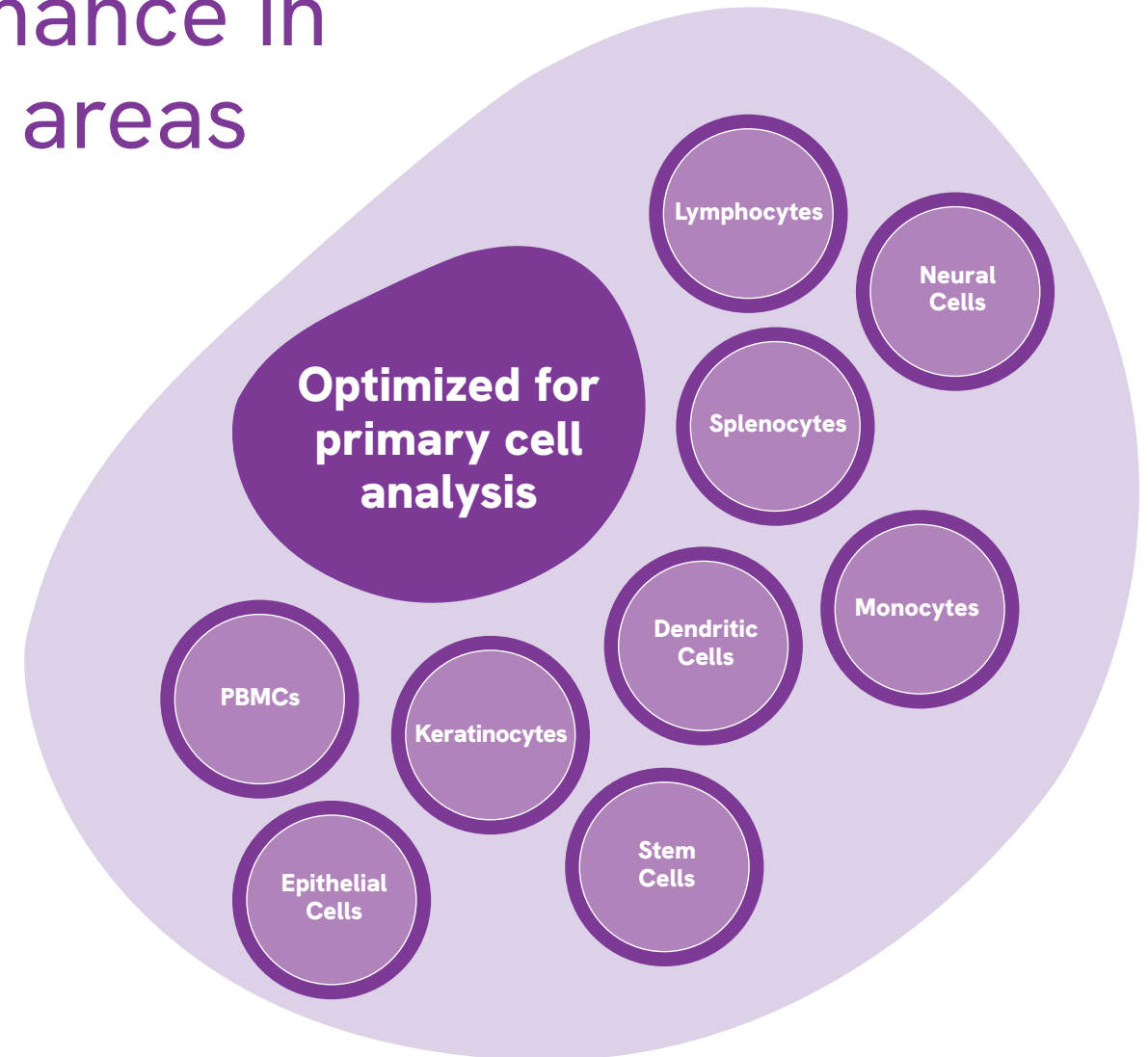


Proven performance in many research areas

- **Clinical Immunology:** PBMCs
- **Regenerative Medicine:** stem cells
- **Transplantation:** nucleated cells
- **Vaccine Development:** splenocytes
- **Oncology:** cell lines
- **Basic Research:** primary cells / cell lines

“My colleague and I purchased a Cellometer Auto 2000 cell counter and we are using it now. It has facilitated our work greatly. We routinely process PBMCs from both fresh whole blood and from frozen stock. The Cellometer has made it much easier to get cell numbers and viability percentages for use in downstream applications such as IVS and Elispot.

- Human Longevity, Inc



Dual fluorescence

Primary cell viability in heterogeneous samples live/dead cell concentration using AO/PI

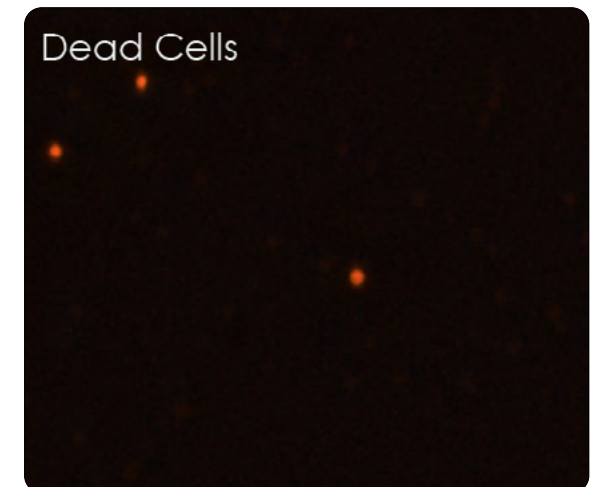
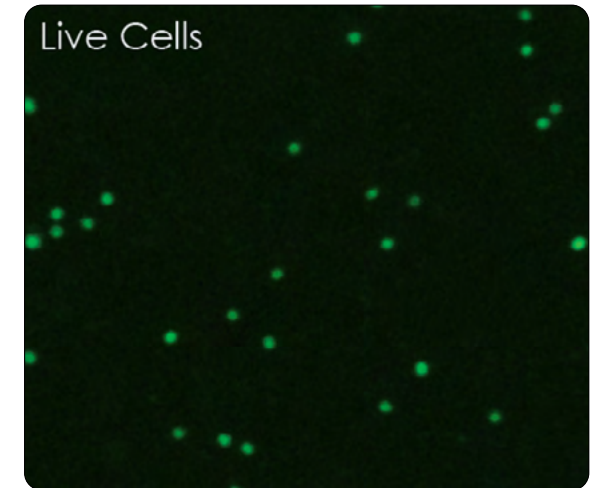
Why isn't Trypan blue recommended for viability analysis of primary cells?

Trypan blue dye enters and stains all cells with a compromised membrane, including both nucleated and non-nucleated cells, such as red blood cells. For the most accurate calculation of nucleated cell viability, fluorescent nuclear staining dyes are required.

Dual-fluorescence viability, using acridine orange (AO) and propidium iodide (PI), is the recommended method for accurate viability analysis of primary cells, such as PBMCs, splenocytes, and stem cells, in samples containing debris and unwanted non-nucleated cell types including red blood cells.

Acridine orange (AO) and propidium iodide (PI) are nuclear staining (nucleic acid binding) dyes. AO is permeable to both live and dead cells and stains all nucleated cells to generate green fluorescence. PI enters dead cells with compromised membranes and stains all dead nucleated cells to generate red fluorescence.

Because mature mammalian red blood cells do not contain nuclei, only live and dead mononuclear cells produce a fluorescent signal. There is no need to lyse red blood cells, saving time and eliminating an extra sample preparation step.



Results that count

The results indicate the accuracy of the Cellometer Auto 2000 instrument in assessing the viability of Jurkat cells using PI for cell viability. Four measurements were performed for each sample. The viability average was calculated and plotted. The results show the reliability and accuracy of the Cellometer Auto 2000 in measuring cell concentration and viability of mammalian cells.

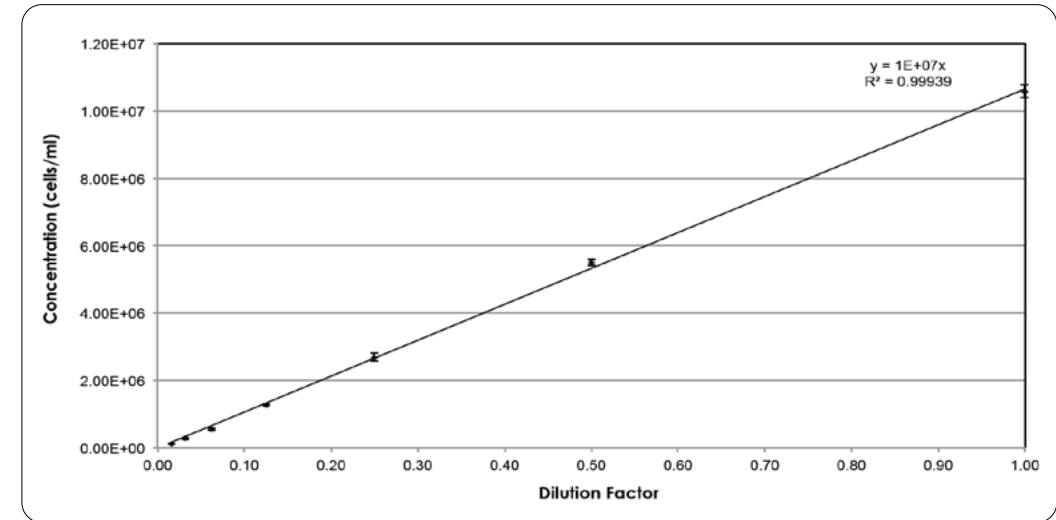


Figure 1: Table of results for cell concentration. Data shown depicts the dynamic range for cell concentration measurements on Cellometer Auto 2000.

The concentration can be measured from 1×10^5 - 1×10^7 cells / mL without further dilution. The %CV at each concentration was below 10%. This data set was taken on a concentration series of primary mouse splenocytes.

Table 1: Results for cell viability using PI only.

Sample	N Value	Average live cell concentration	% Viability	CV of concentration	CV of viability
A	4	4.20E+06	91.1	10%	2%
B	4	1.06E+06	22.7	7%	1%
C	4	3.27E+06	57.5	7%	7%

Features

The screenshot shows the Cellometer Auto 2000 software interface. At the top, it displays 'Cellometer Auto 2000'. Below this, there are buttons for 'Edit Assay', 'Import Assay', 'Sample ID: new sample', and 'Dilution Factor: 2.00'. The main area is titled 'Assays Available for Selection' and contains several assay cards:

- BR/Green/Red** Current Assay: Primary cells, cell lines
- BR/Green/Red** Stem cells: AOPI (CS2-0106) or equivalent, Stem cell sample
- BR/Green** GFP Efficiency and Viability: GFP/PI (CS1-0100), GFP Transfection Efficiency and Percent Viability
- Green/Red** Immune cells, high RBC: AOPI (CS2-0106) or equivalent, Nucleated cells in samples with large amount of red blood cells. No RBC lysis.
- BR/Green/Red** Immune cells, low RBC: AOPI (CS2-0106) or equivalent, Nucleated immune cells after isolation in samples with some red blood cells. PBM C after ficoll separation, splenocyte without trying H&C.
- BR** Cell line, viability trypan blue: Trypan blue, Cell line or cultured primary cells without debris.
- BR/Green/Red** Primary cells, cell lines: AOPI (CS2-0106) or equivalent, Primary cells, cell lines or cell sample from dissociated tissues with debris.

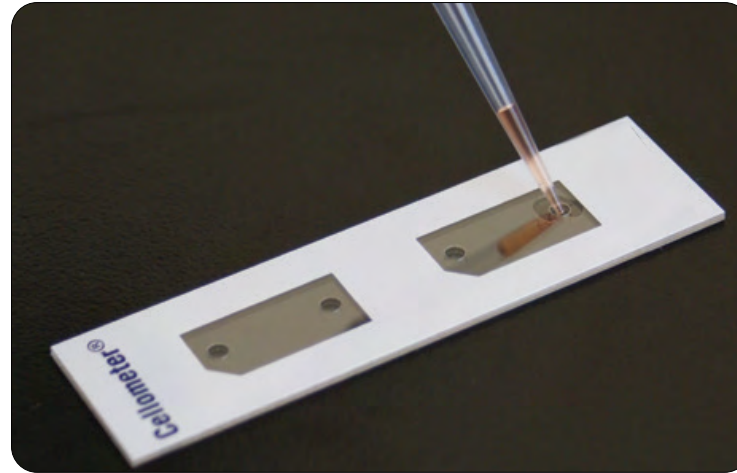
At the bottom, there are buttons for 'Preview Image for Current Assay', 'Load Image', 'Edit Instrument Settings', and 'Access User Help'. A 'Cell Diameter Histogram' is shown in the top right corner, displaying a bar chart of cell sizes.

Callouts and Features:

- Analysis of multiple species of stem cells:** Points to the 'Stem cells' assay card.
- Easily edit and import assays:** Points to the 'Edit Assay' and 'Import Assay' buttons.
- User-friendly touch screen:** Points to the overall interface.
- Total nucleated cell count & viability:** Points to the 'Immune cells, high RBC' assay card.
- One-Step cell concentration and viability:** Points to the 'Cell line, viability trypan blue' assay card.
- Images for data verification:** Points to the 'Preview Image for Current Assay' button.
- Cell size histograms:** Points to the 'Cell Diameter Histogram' chart.
- GFP transfection efficiency and % viability:** Points to the 'GFP Efficiency and Viability' assay card.
- Primary splenocyte concentration & viability:** Points to the 'Immune cells, low RBC' assay card.
- Accurate PBMC counts in the presence of red blood cells:** Points to the 'Immune cells, low RBC' assay card.

How it works

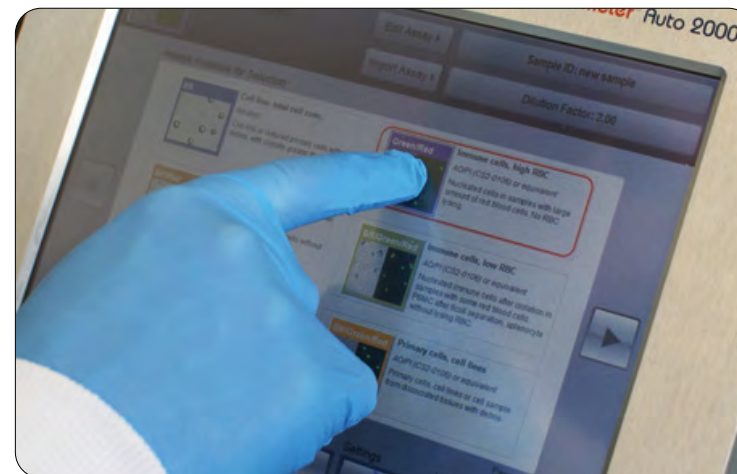
Step 1: Pipette 20µl



Step 2: Insert counting chamber



Step 3: Select assay and click count

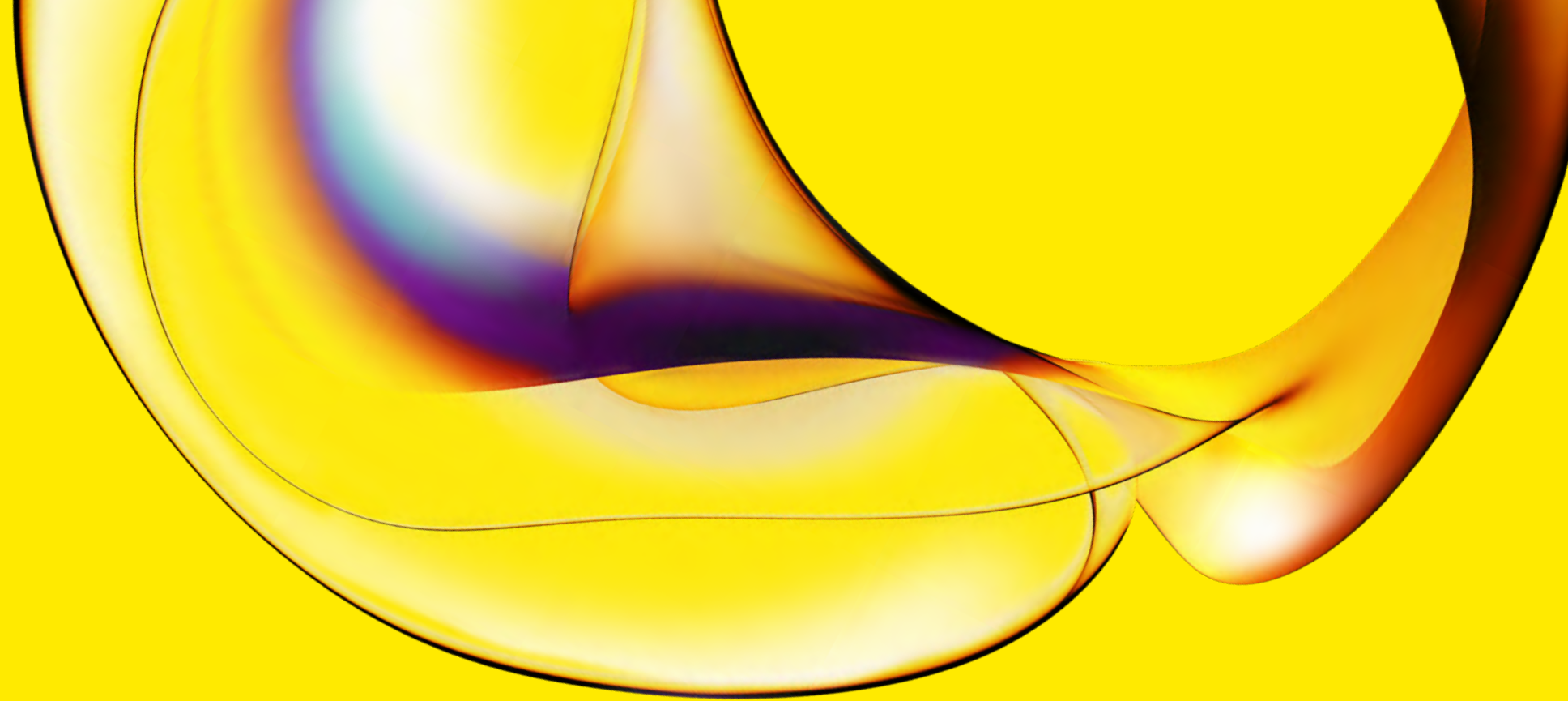


Step 4: Get results

Assay: Immune cells, high RBC

Sample ID: Blood_AOPI_4-2
Dilution Factor: 2.00

Count	Concentration
===== Total: 340 cells	===== 1.18x10 ⁶ cells/mL
Live: 324 cells	1.12x10 ⁶ cells/mL
Dead: 16 cells	5.53x10 ⁴ cells/mL



www.revivity.com

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Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revivity.com

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