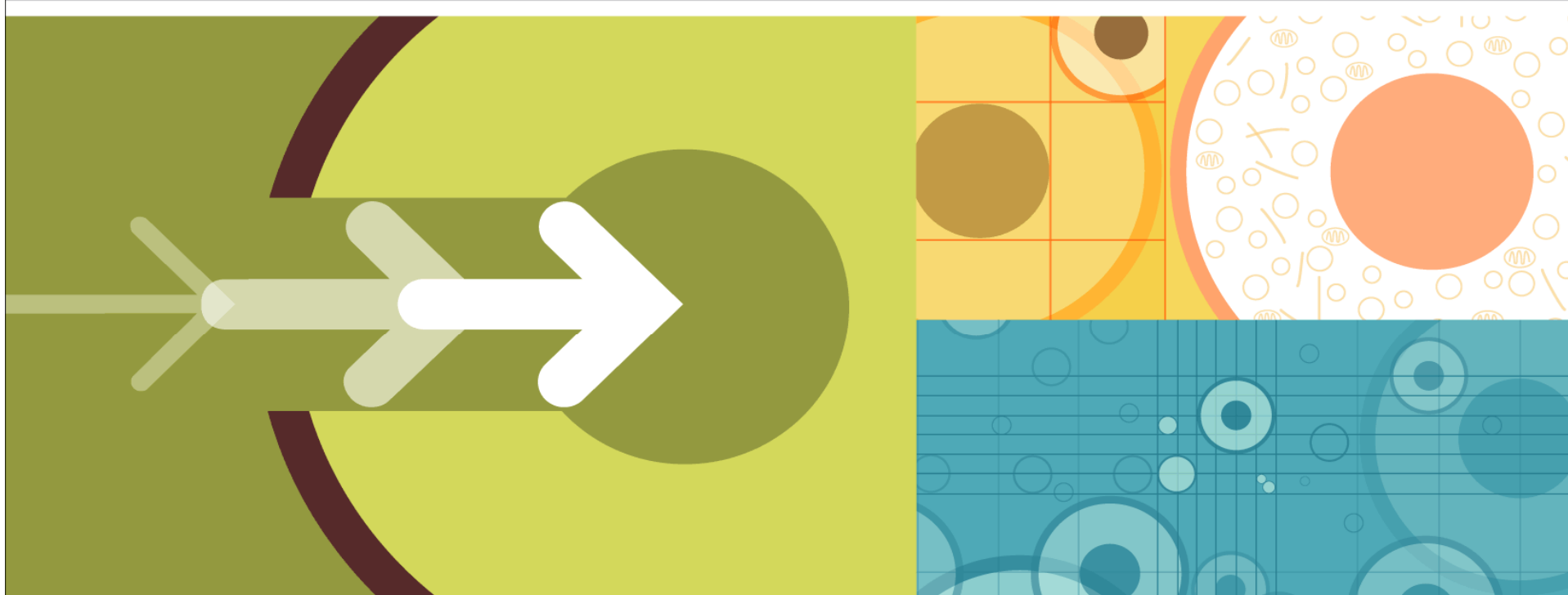
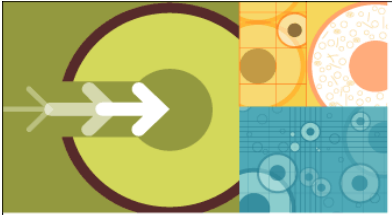


TC10 Automated Cell Counter



Cellular Systems

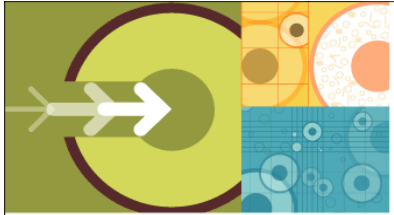
BIO-RAD



Cell Counting Methods

- Hemocytometer
- Dedicated (non-flow) cell counters
 - Remove the tedium of manual counting with a hemocytometer, but they have limitations
- Flow cytometers
 - Not designed for cell counting
- Coulter counter (impedance based)
 - Hematology laboratories

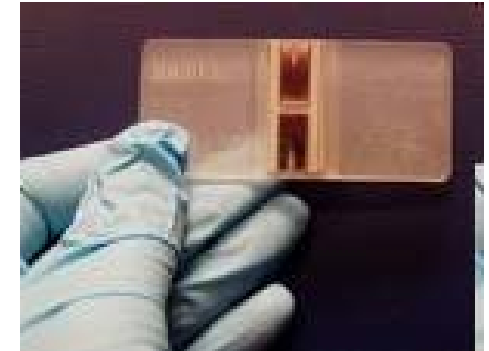




Cell Counting with a Hemocytometer

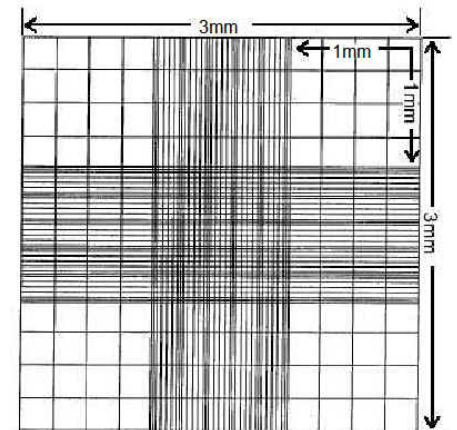
- Workflow

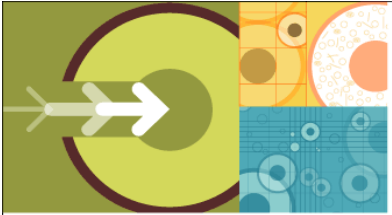
- Pipet cell suspension into a hemocytometer slide, manually count the cells within an area, calculate concentration and then the dilution needed for the next passage or the experiment



- Problems

- Tedious
- Slow (30-120 sec per count), plus time for calculations
- Limited cell concentration range
- Subject to bias, results are user-dependent and vary among researchers

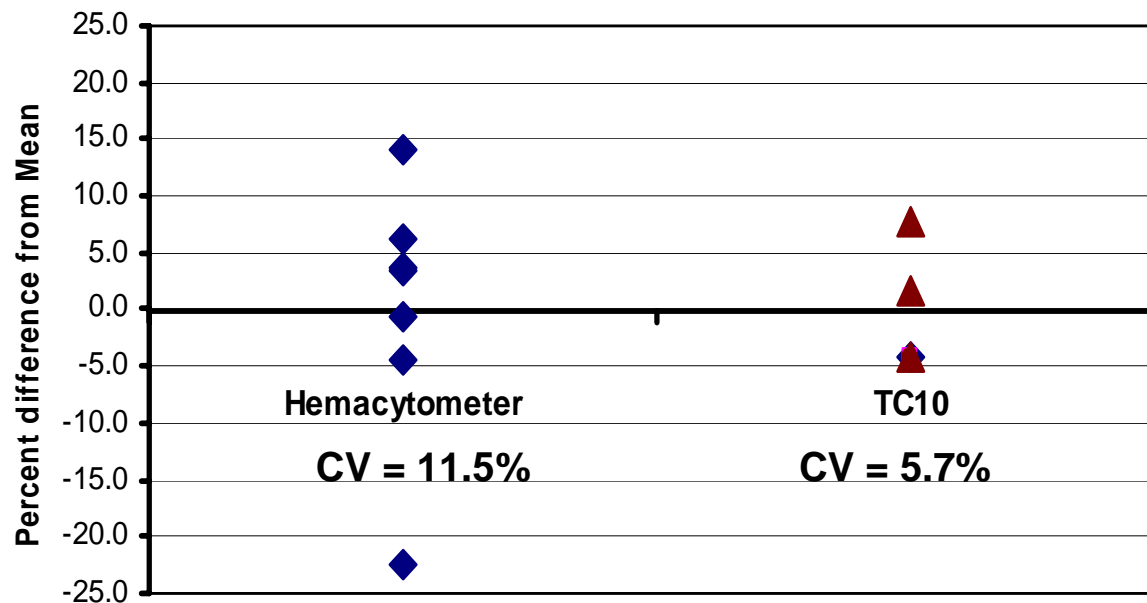


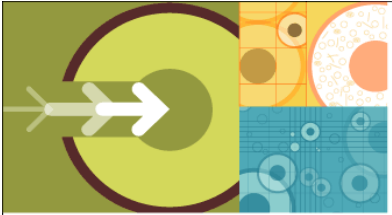


Counting Variation - TC10 vs. Hemocytometer

“During beta test I found large variations between 4 hemocytometers in our lab and between people in the lab. I do not trust hemocytometer counts too much anymore and I wonder if counting might be a big source of error in the laboratory.

Manual vs TC10 counts variation

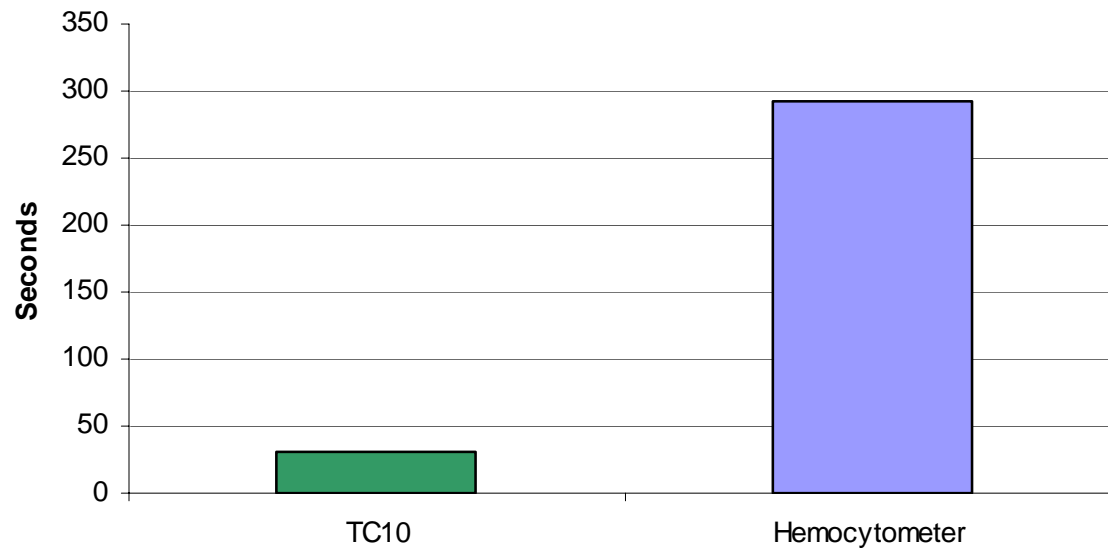




Time Savings TC10 vs. Hemocytometer

TC10 is much faster, 18 counts in 11 minutes versus only 4 with hemocytometer during same time period

Comparison of time spent counting cells with a TC10 versus hemocytometer



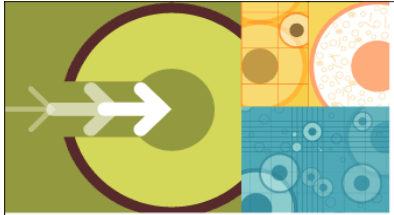


TC10 Automated Cell Counter

The truly automated cell counting solution

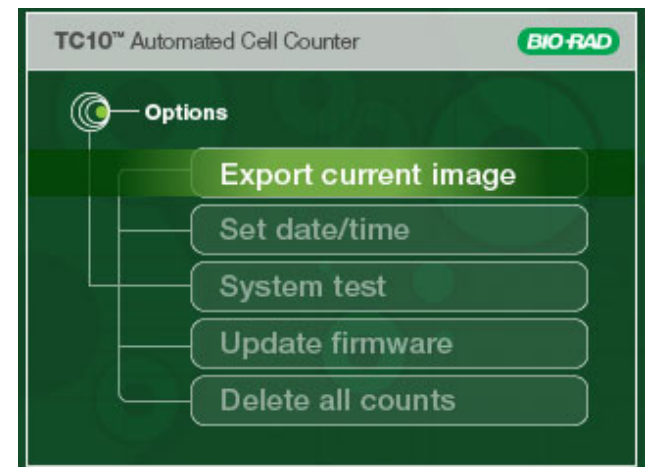
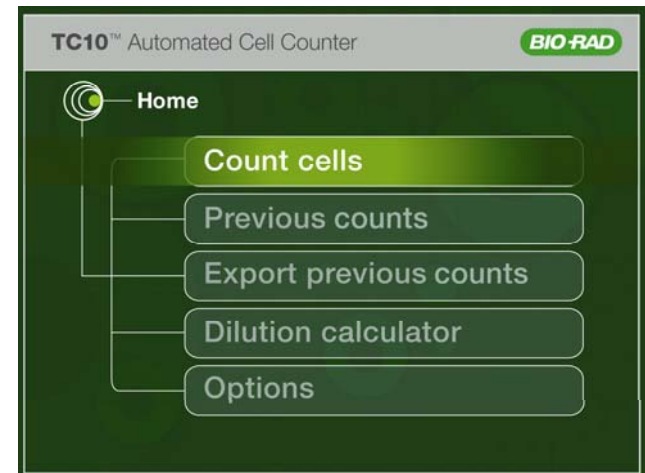
- **Fit cell counting into your schedule** — count cells quickly, accurately, and consistently within 30 sec using the built-in auto-focus
- **Configure results to your needs** — determine total cell count without dye or use Trypan blue dye to assess total cell count and cell viability
- **Have results at your fingertips** — print count results and dilution calculations using the TC10 label printer
- **Easily archive your results** — transfer counts and cell images using a USB key or access up to 100 counts stored in the onboard memory
- **Trust your counts** — confirm instrument functionality with the TC10 verification slide
- **Conserve precious cells** — use only ~10 μ l of suspended cells

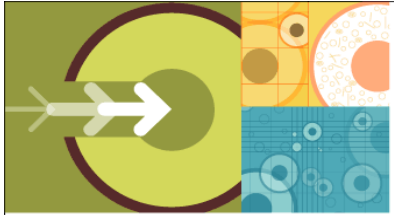




Easy User Interface

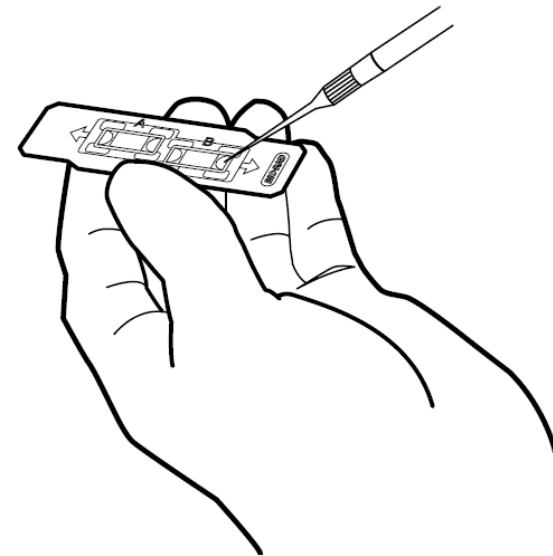
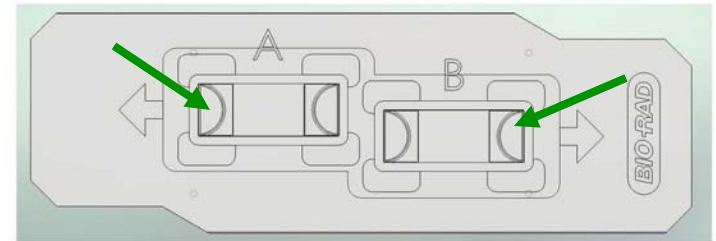
- 4 keys for navigation
- The Home screen is the starting point for using the TC10
 - **Count cells** — initiates a cell count
 - **Previous counts** — for viewing results from up to 100 previous counts stored in the instrument
 - **Export previous counts** — for exporting results onto a USB flash drive
 - **Dilution calculator** — calculates volume adjustments for the next passage/experiment
 - **Options** — for selecting additional operations

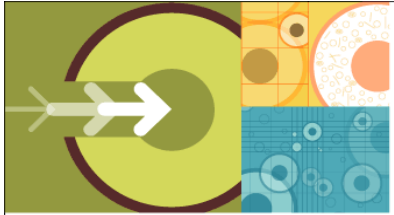




Preparing Samples

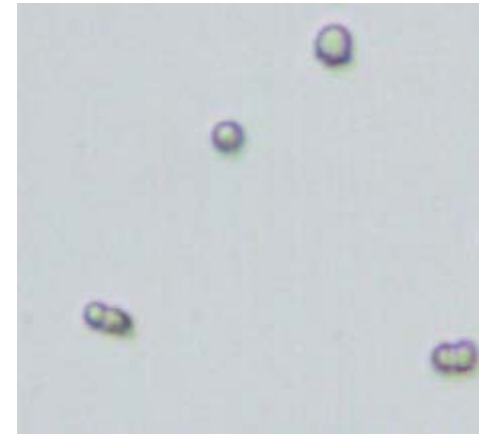
- Dual-chamber slides accommodate two samples (A and B)
- Load sample into half-moon opening on **outer** side of the counting chamber
 - Capillary action and slide design ensure even sample distribution in the chamber
- For total count, load 10 μl of cell suspension
- For total count and live count, create a 1:1 mixture of cell suspension with Trypan blue dye and load 10 μl



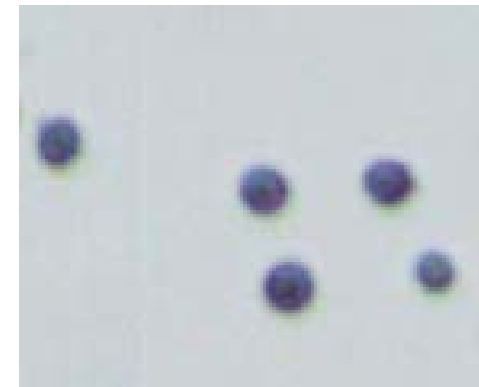


Determining Cell Viability

- The TC10 measures cell viability via Trypan blue exclusion
 - The Countess requires Trypan blue for all counts, including just the total count
- **Live cells** - intact cell membrane prevents the Trypan dye from entering, live cells remain bright
- **Dead cells** - compromised cell membrane can not stop Trypan dye from entering, and the cells are blue



Live cells



Dead cells



Counting Cells

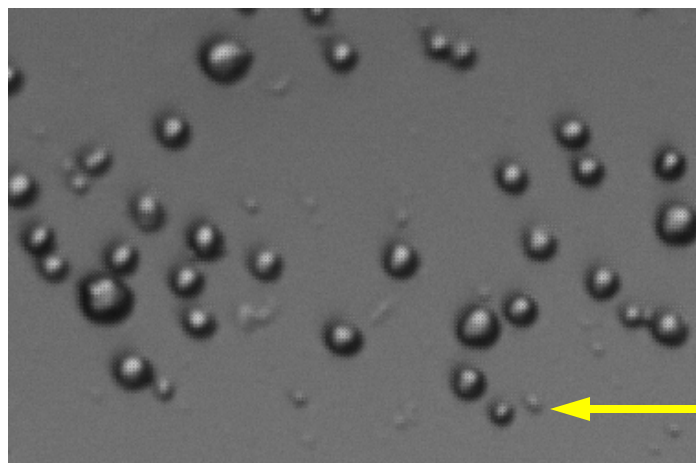
- TC10 initiates a count upon slide insertion
 - Optical switch initiates the count
 - No user interaction needed to start the count
- TC10 detects automatically the presence of Trypan blue in the sample, and provides a total and live cell count
- Typically counts in under 30 seconds
 - Cell concentrations above 8×10^5 cells/ml can take up to 45 seconds



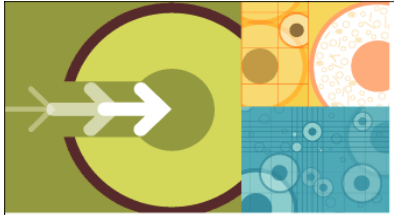


How the TC10 Counts Total Cells

- TC10 uses light microscopy and digital image based counting
- The TC10 uses a unique autofocus technology, eliminating the need to manually focus
 - Determines the best focal plane for discrimination of cells against the background and any debris
 - Acquires an image from the best focal plane
 - Counting algorithm analyzes the digital image for total cell count



Debris



How the TC10 Counts Live Cells

- The TC10 uses a multi-focal plane analysis for live/dead cell count (samples with Trypan blue)
- The total cell count is obtained from the best focal plane
- Counting of live and dead cells requires analysis of multiple focal planes
 - Light scattering and alignment of cells at different heights in the counting chamber cause some live cells to appear dead if analyzed only on 1 focal plane resulting in live cells undercount
 - Each cell is analyzed on multiple to determine if it live or dead

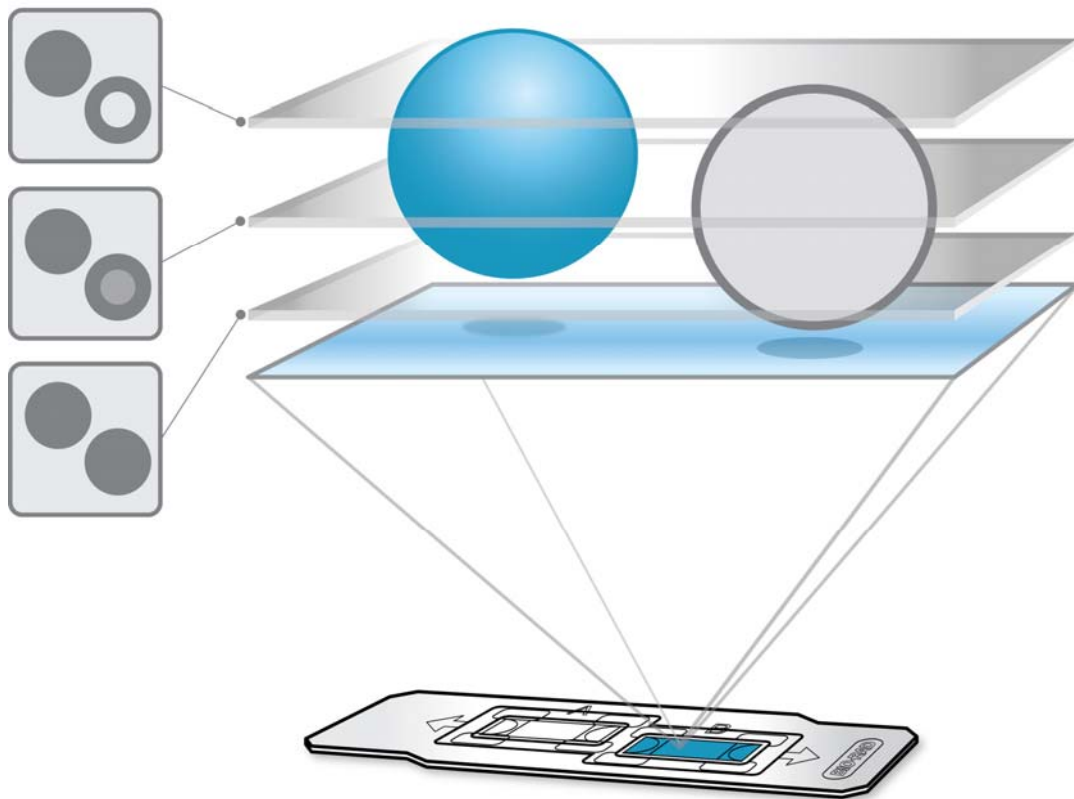


Best focal plane

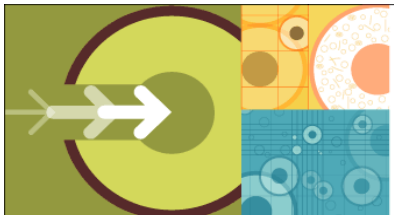


Multi-Focal Plane Analysis

Top View



- Resonates with microscope and hemocytometer users
- During microscope focusing user goes through multiple focal planes and some live cells can appear as dead at certain focal planes



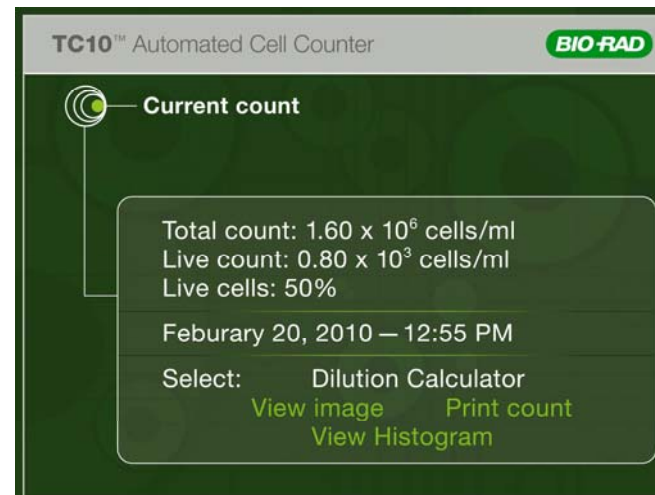
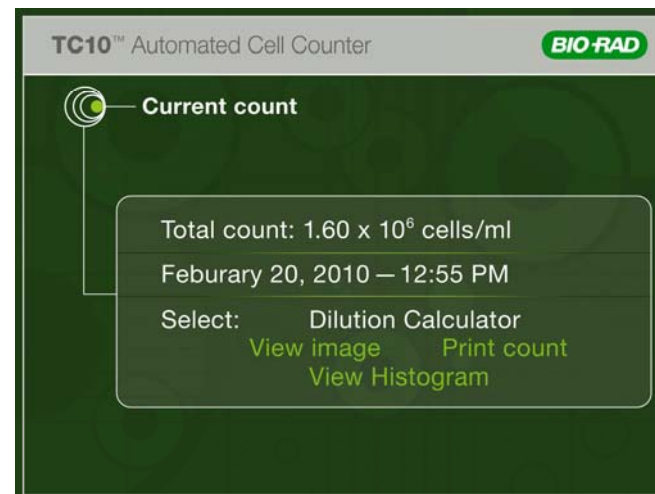
Multi-Focal Plane Analysis for Viability Determination

HeLa cells	% of live cells	% of dead cells	Total cells/ml
Cell viability (manual count)	65%	35%	2.5×10^5
Cell viability from single best plane	35%	65%	2.5×10^5
Cell viability from multiple planes	65%	35%	2.5×10^5



Count Results

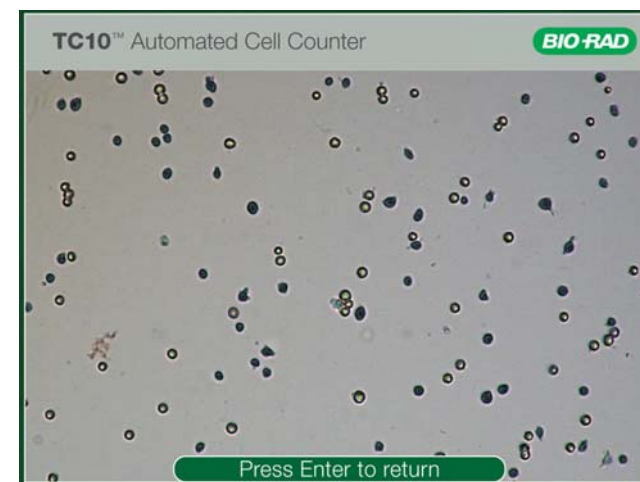
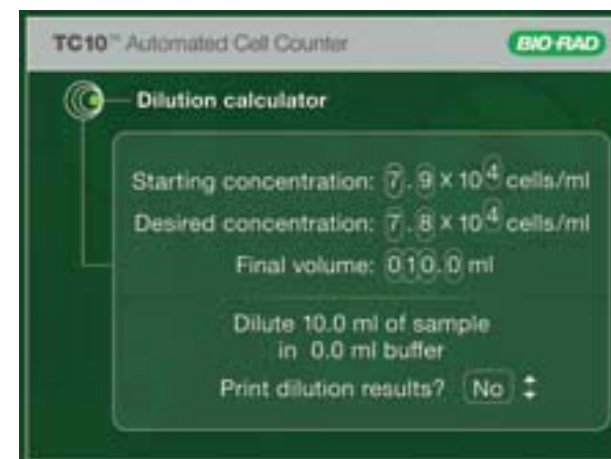
- Results without Trypan blue
 - Total cell count per ml
- Results with Trypan blue
 - Total cell count per ml
 - Live cell count per ml
 - Live cell percentage
 - TC10 takes into account the 1:1 sample dilution with Trypan blue

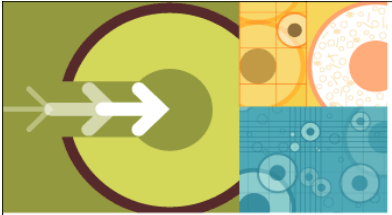




Analysis Options

- Dilution calculator
 - Calculates sample adjustments needed for next experiment or passage
- View image
 - Check for cell clumps or use when becoming comfortable with the instrument
 - Zoom in and out with Up/Down keys

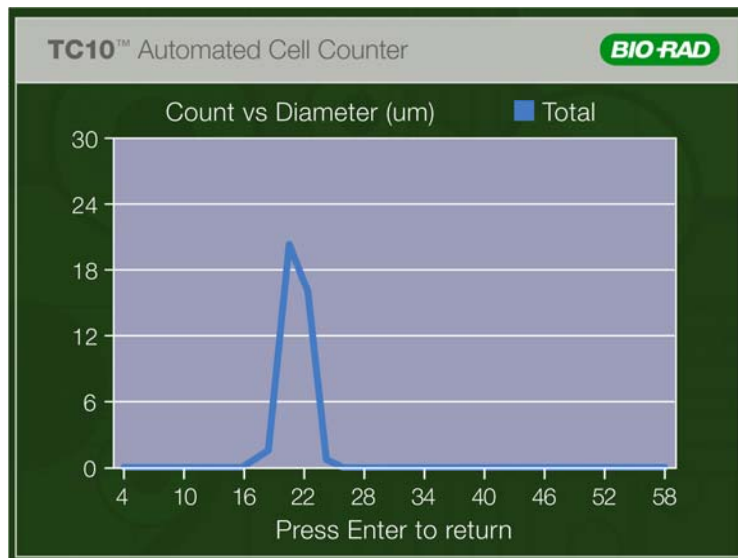




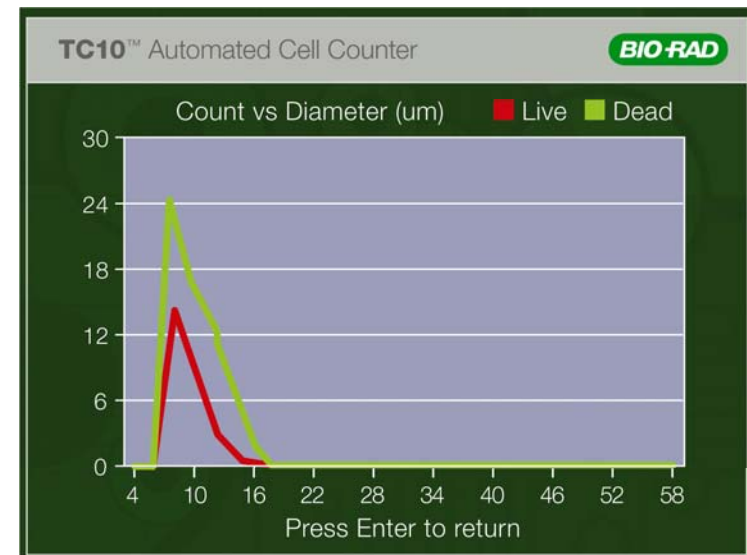
Analysis Options

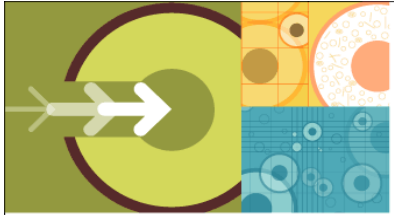
- View histogram
 - Histogram of cell size distribution within the imaged area (4 mm²)
 - Approximate cell diameters in μm

Sample without trypan blue



Sample with trypan blue

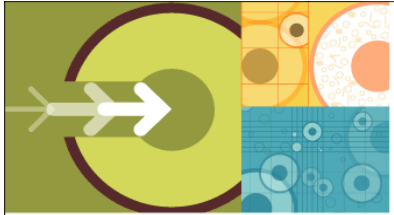




Printing Results

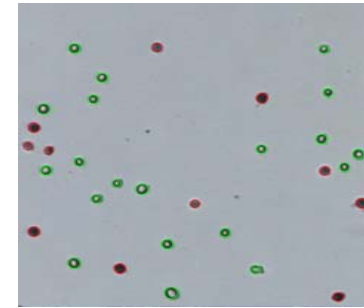
- Print onto labels using the TC10 thermal printer
 - Current count
 - Any previous count
 - Dilution calculation
- Hook up the printer directly to the TC10 cell counter via the USB port (A or B)
- Printing requires firmware and only this printer communicates with the TC10
- For count traceability, the labels can be placed directly into the lab notebook





Data Storage and Exporting

- Image from the most recent count
 - Export to a USB key (USB port A)
 - Annotated JPEG file
 - Result and time stamp are in the image
- 100 previous counts are stored in the TC10
 - Previous count data can be exported on a USB drive and opened in Excel
 - Counts organized by date and time stamp
 - Counts can be deleted for easier identification of results among multiple users



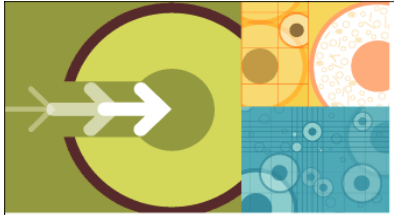
TC10 Automated Cell Counter
Export Results
Firmware Version: 1.080
December 15, 2009 - 10:49

Result 1 of 17
December 10, 2009 - 14:53
Total cell count: 1.4×10^5 cells/ml

Result 2 of 17
December 10, 2009 - 15:02
Total cell count: 1.6×10^5 cells/ml
Live cell count: 1.6×10^5 cells/ml
Live cells: 100%

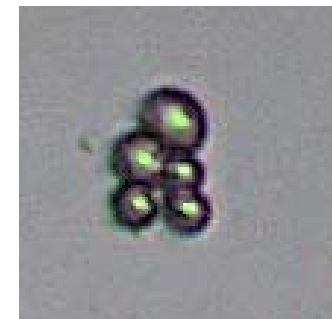
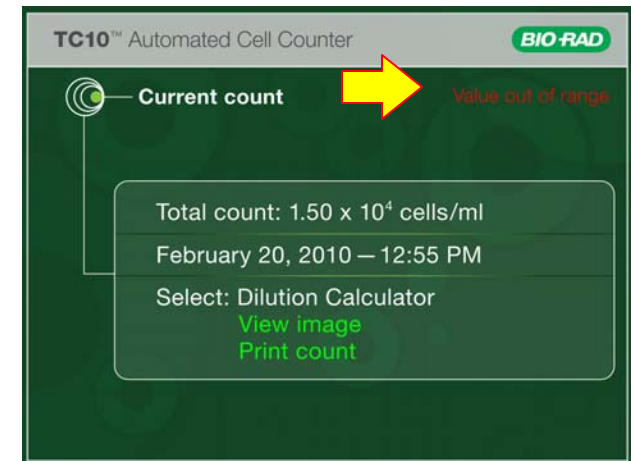
Result 3 of 17
December 10, 2009 - 15:09
Total cell count: 5.9×10^5 cells/ml

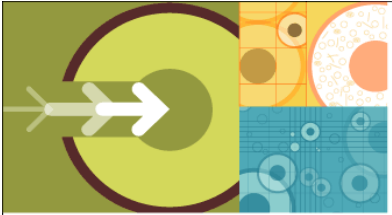
Result 4 of 17
December 10, 2009 - 15:14
Total cell count: 8.7×10^5 cells/ml



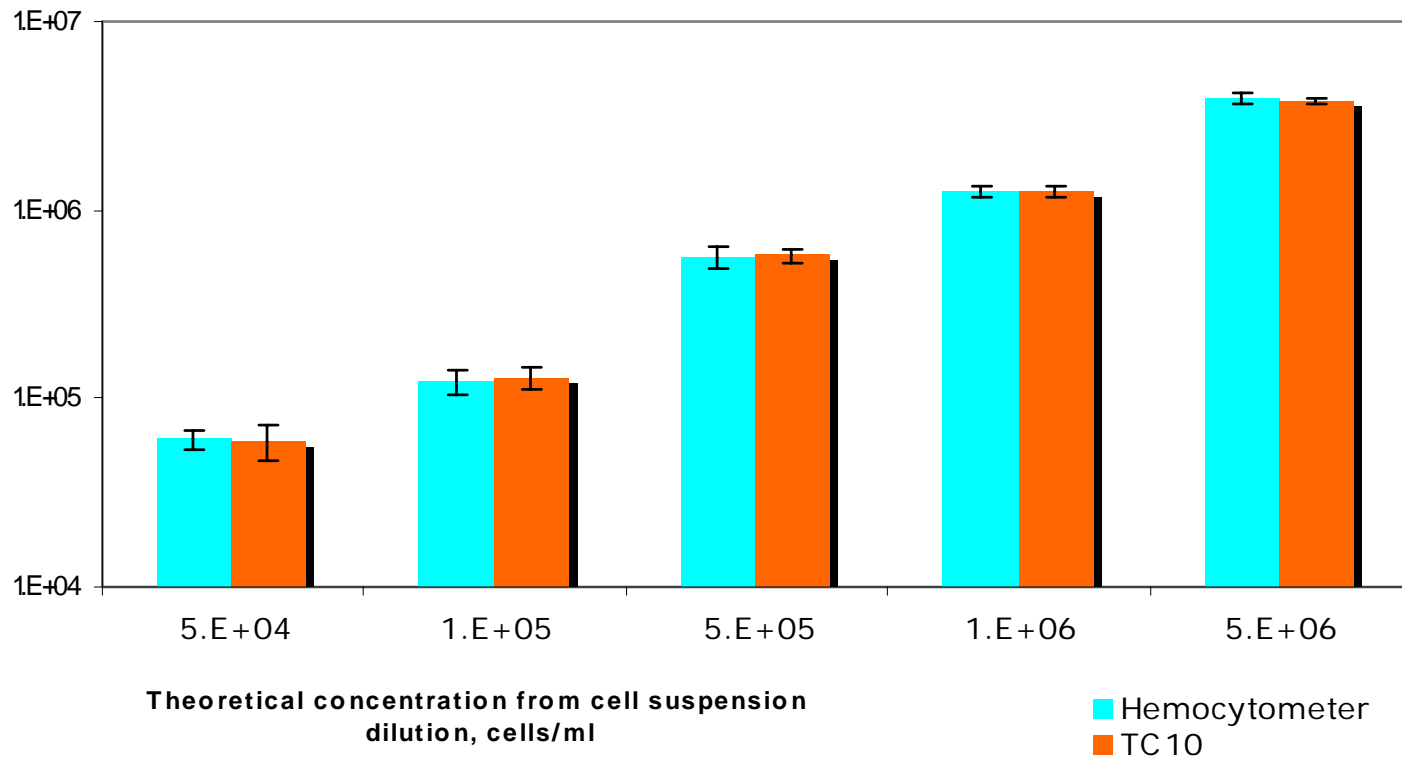
Counting Specifications

- Concentrations: 5×10^4 and 1×10^7 cells/ml
 - If out of range, a message alerts the user
- Cell diameter between 6 - 50 μm
- Cell clusters
 - Cells naturally create clusters
 - Break apart with trypsinization or mechanically by pipetting
 - TC10 distinguishes individual cells in clusters of up to 5 cells





Performance Data



TC10 demonstrates accurate cell counts across an extended range of cell concentrations. MEF cells were concentrated, serially diluted and counted with a hemocytometer and TC10 cell counter. The TC10 cell counter and hemocytometer cell counts do not show statistical difference. Cell counts on the TC10 cell counter were performed on 4 different instruments, with 6 sample replicates. Error bars indicate precision.



Ordering Information

- 145-0001 - TC10 Automated cell counter
 - Includes instrument, 30 dual chamber counting slides, 1.5ml of Trypan blue dye
- 145-0009 - TC10 Automated cell counter with printer
 - Includes instrument, label printer, 30 dual chamber counting slides, 1.5ml of Trypan blue dye
- 145-0003 - TC10 Counting kit
 - Includes 30 dual chamber counting slides, 1.5ml of Trypan blue dye
- 145-0014 - TC10 Verification kit
 - Includes TC10 verification slide and verification protocol to validate TC10 functionality
- 145-0011 Thermal label printer
- 145-0007 Printer labels

