Technical Overview

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Automated Cell Counting with High Contrast Brightfield and EVE Counting Slides

Introduction

The Agilent BioTek Cytation 1 cell imaging multimode reader is a versatile instrument for conducting a broad range of cell-counting applications. High-contrast brightfield imaging and Agilent BioTek Gen5 microplate reader and imager software deliver convenient and accurate label-free cell counts, with calculated cell concentration and cell size profiles reported. The ability to use up to four fluorescent imaging cubes, available in a broad range of wavelengths, enables detailed characterization of cell viability using diverse fluorescent markers. Disposable EVE counting chambers from NanoEnTek (Seoul, Korea) eliminate the need for washing components, increasing productivity and reducing user exposure to hazardous samples compared to a conventional hemocytometer.

Assay overview

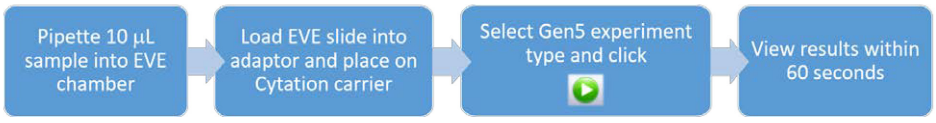

Figure 1. Assay flow chart.

Automated cell counts with Cytation and EVE counting slides

Each EVE slide contains two independent counting chambers. The Cytation slide adapter (part number 1220548) accommodates two EVE slides for measuring up to four samples in approximately 60 seconds. The automated protocol collects four images with a 4x objective and stitches them together to produce a large area per chamber (11.5 mm^2) for accurate determination of sample concentrations.

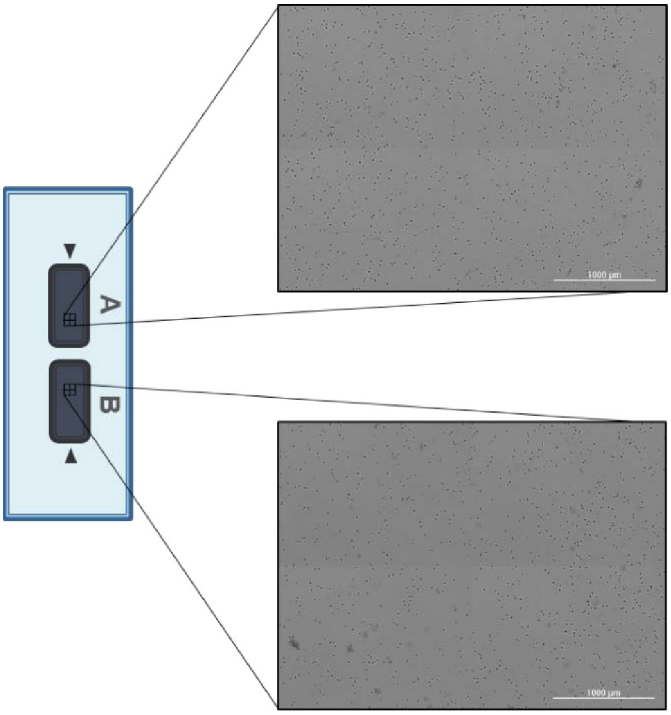

Figure 2. Model of an EVE counting slide depicting imaging area and representative images captured of each chamber. A large image area results in more reproducible cell counts and accurate determination of sample concentration.

Accurate and robust cell counts with high-contrast brightfield imaging

Gen5 image analysis software automatically identifies cells from high-contrast brightfield images based on size, circularity, and intensity thresholds. The software is able to resolve individual cells within clusters and generate accurate counts of samples containing debris. Irregular-shaped cells can be identified and counted by adjusting the circularity threshold or defining subpopulations. Cell morphology and accuracy of cell counts can be evaluated by viewing high-resolution images.

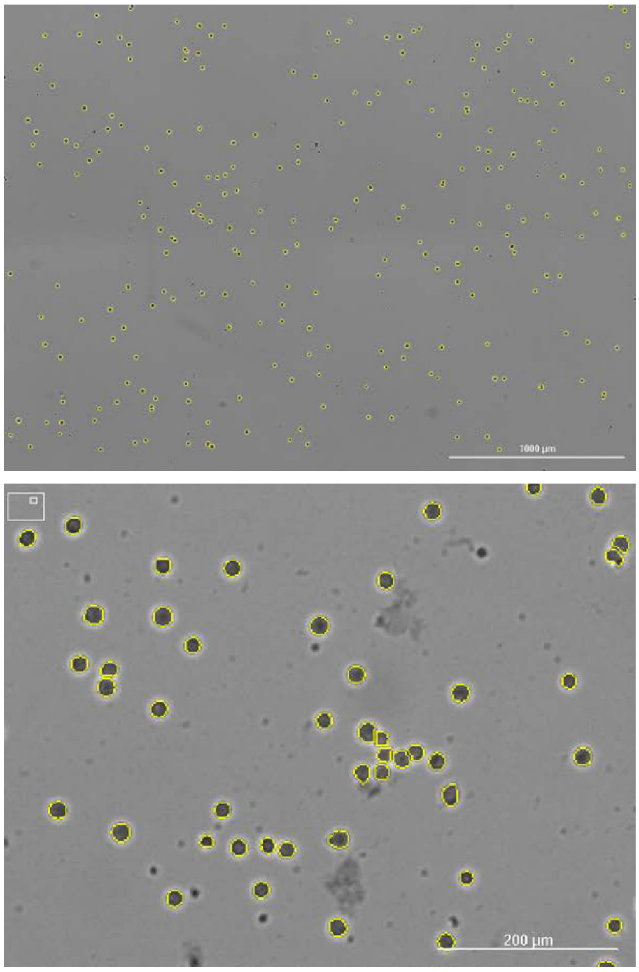

Figure 3. Cell counts are determined from high-contrast brightfield images and Agilent BioTek Gen5 microplate reader and imager software. Accuracy of cell counts are easily verified by viewing images with masks placed around each cell. Gen5 uses advanced image analysis features to resolve individual cells within clusters while ignoring debris.

Automated image-based counts result in less variation compared to manual methods

Manual cell counting with a hemocytometer depends on subjective evaluation by the user, leading to erroneous and inconsistent results.

The Cytation system uses Gen5 software to conduct automated image capture and analysis, minimizing subjectivity and generating cell counts in less time with increased reproducibility across users.

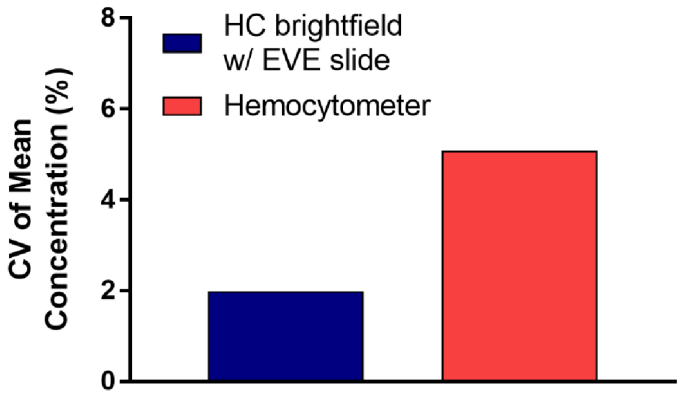

Figure 4. Cell counts of HEK293 samples were conducted by four different operators using both a manual hemocytometer and an Agilent BioTek Cytation 1 cell imaging multimode reader with EVE counting chambers. Considerably less variation across counts was observed with the Cytation.

Cell size determination and subpopulation analysis

The Gen5 histogram feature produces a detailed description of cell size for each sample with mean and variance reported. The cell size profile from multiple samples can be viewed simultaneously for comparative analysis. Subpopulations can be defined in Gen5 using cell characteristics of interest and quantified for each sample.

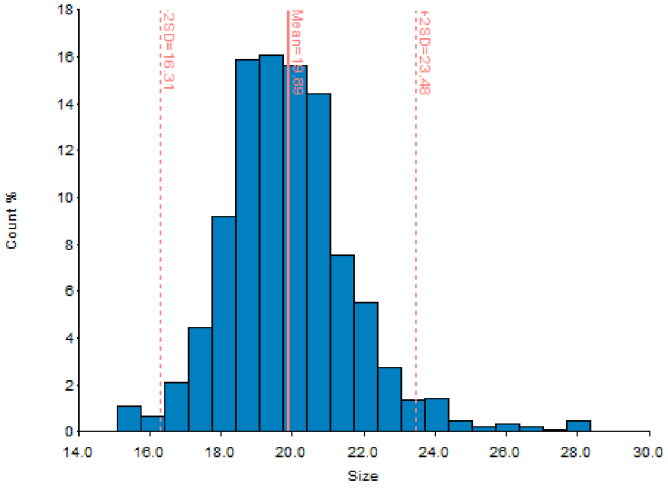

Figure 5. Histogram of HEK293 sample cell size.

Analysis of cell viability

The Cytation 1 cell imaging multimode reader is available with a broad range of imaging LED and filter cubes for cell-counting applications involving fluorescent markers designed to measure cell viability. Percent live/dead calculations and subpopulation analysis are performed automatically based on preset parameters.

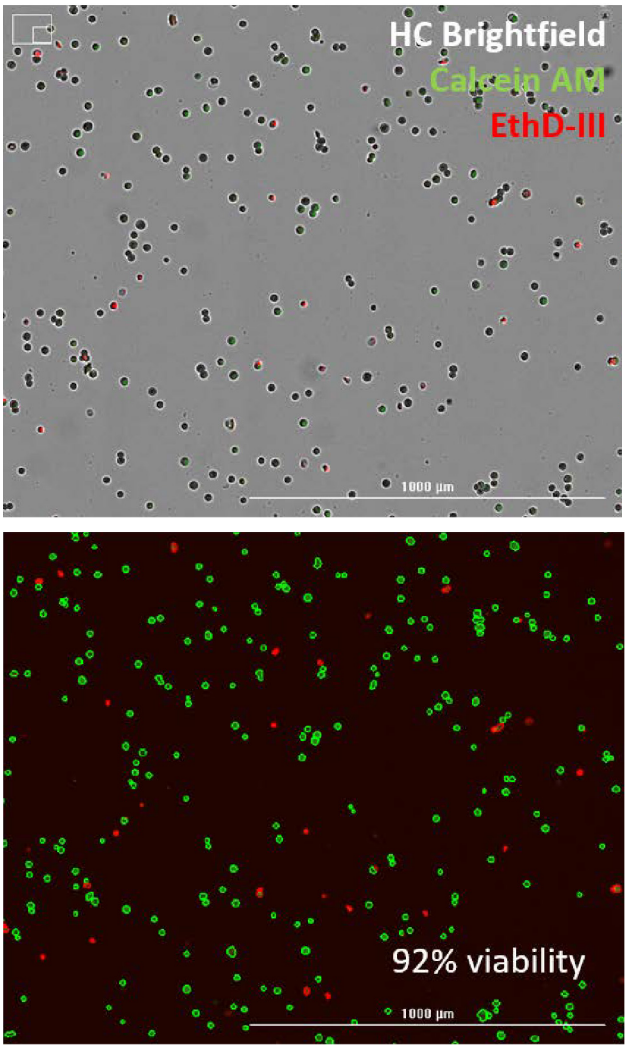

Figure 6. Calcein AM is an esterase substrate that stains live cells green, while ethidium homodimer-III (EthD-III) is a membrane-impermeable DNA dye that stains dead cells red. When these two stains are used in conjunction, they provide a sensitive dual-fluorescence system for conducting automated cell viability measurements.

Conclusion

Available high-contrast brightfield imaging on the Agilent BioTek Cytation 1 cell imaging multimode reader provides accurate label-free cell counts for adherent and suspension cell types. The broad range of imaging LED and filter cubes enable fluorescence-based characterization of cell viability and subpopulation analysis. Agilent BioTek Gen5 microplate reader and imager software automatically calculates concentration, cell size, and percent viability. Disposable EVE chamber slides provide a convenient and economical cytometer for use with the Cytation. Combined, the Cytation 1 and EVE counting chambers deliver a fast and reliable solution for conducting diverse cell counting applications.

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