Control Strategies for Improving Confidence in Dye Exclusion-Based Cell Viability Measurements

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Cell viability assays are critical in cell therapy manufacturing and product release. In recent years, imaging aspects of cell viability assays have become automated; however, acquisition and analysis steps in automated viability assays can introduce variability that should be controlled to ensure image measurement quality. Here, we describe two novel techniques developed to improve the reliability cell viability measurements: 1) a semi-automated trypan blue cell viability assay which implements a bead-based spike-in control material to benchmark the image focal plane of brightfield images, and 2) an image-based fluorescence dye exclusion viability assay which uses assigned equivalent reference fluorophore (ERF) value fluorescent microspheres to enable transfer of intensity scale and gating of fluorescent channels, and to enable improved thresholding for quantitative comparison of pixel intensities. Both techniques work to establish improved methods for classifying live and dead cells.

Methods: Jurkat cells (Jurkat, Clone E6-1, ATCC TIB-152) were used in this study. Bangs 100% ViaCheck viability control beads (Bangs Labs, VC50B) were used to benchmark image focus. Spherotech 6-peak and 9-peak ultra rainbow calibration particles (Spherotech URCP-100-2 and URCP-100-2H) were used to benchmark fluorescence intensity. Bead and cell imaging studies were conducted using the Nexcelom Cellometer Auto 2000 (Trypan blue measurements), and the Chemometec NC-3000 and a Nikon Ti2 Eclipse widefield epifluorescent microscope (fluorescent measurements). For brightfield and trypan blue studies, data containing a full focal range sweep were acquired in five separate studies conducted on different days for replication. Beads were detected in images by segmenting, applying morphological operations and filtering based on bead properties. For percent viability recovery studies, non-viable cells were generated via heat shock treatment to generate samples with approximate ratios of viable and non-viable cells. For fluorescent intensity benchmarking studies, 6-peak and 9-peak Spherotech beads were imaged on the NC-3000 and Nikon microscopy systems and thresholding and pixel intensity quantification techniques were performed in Image J. An ERF value to fluorescence intensity calibration scale was then derived for each microscope and fixed and live cells were imaged using identical imaging conditions to determine relative intensity levels of cells to beads.

Results: Trypan blue viability measurements can be greatly affected by image focal plane (**Fig. 1**). For example, in a 20% viability sample, measured viability ranged from 20% to 86% depending on the focal plane of data acquisition. Cell viability data at the reference focal plane was acquired using the bead benchmarking and reference focal system and an experimental spike-in design using fully heat-killed cells to assess proportionality of the novel system. For fluorescent bead benchmarking, a calibration curve correlating fluorescence intensity of the ERF microspheres to the assigned ERF value was established using the NC-3000 imaging system and the Nikon Ti2 microscope. Based on the linear fit, relative fluorescent intensity values were assigned and compared for fixed and live cell samples using acridine orange as a nuclear cellular stain.

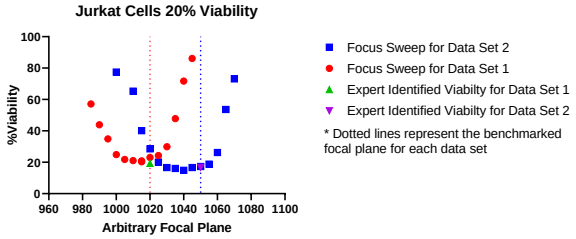
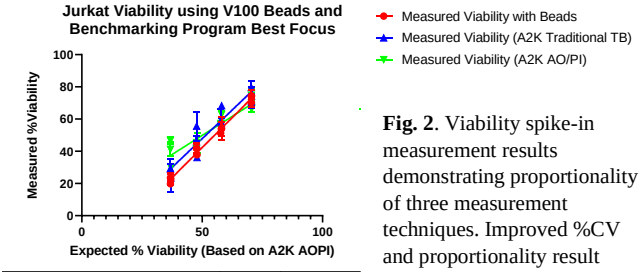


Fig. 1. Viability results as a function of focal plane for two independently acquired data sets. Expert determined cell viabilities (triangles) are represented as well as the benchmarked focal plane (vertical lines).

Conclusions: We demonstrate that by capturing images at a reproducible reference focal plane and with consistent signal-to-noise ratio, improvements in the variability and accuracy of the trypan-blue based cell viability measurement can be achieved (**Fig. 2**). Using ERF beads with a fluorescent image quantification and benchmarking system, we establish that the gating of fluorescent intensity values can be transferred between instruments and that fluorescent intensity results for cells can be compared across microscopes.



	A2K with Beads	A2K TB	A2K AO/PI
Goodness of Fit			
R squared	0.9893	0.9122	0.8528
Sy.x	2.116	6.025	5.354

Fig. 2. Viability spike-in measurement results demonstrating proportionality of three measurement techniques. Improved %CV and proportionality result from using the bead benchmarking program for trypan blue measurements.

References:

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2. Pierce L, Sarkar S, Chan L, Lin B, Qiu J. Outcomes from a cell viability workshop: fit-for-purpose considerations for cell viability measurements for cellular therapeutic products. Cell & Gene Therapy Insights 2021; 7(4), 551-569. DOI: 10.18609/cgti.2021.076