SCIENTIFIC POSTER

Alternative cell counting and viability detection using NexGreen/PI™ fluorescent stain on multiple low and high-throughput image cytometry platforms.

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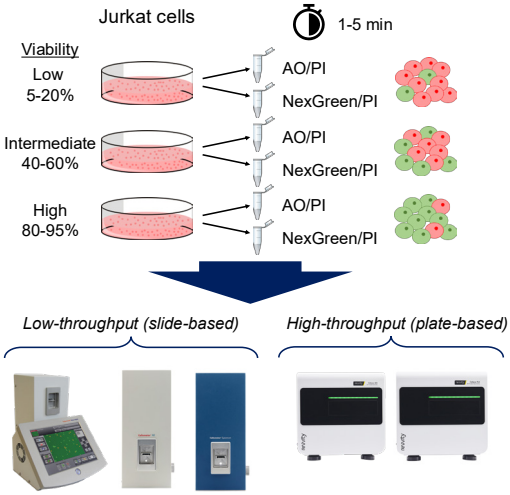
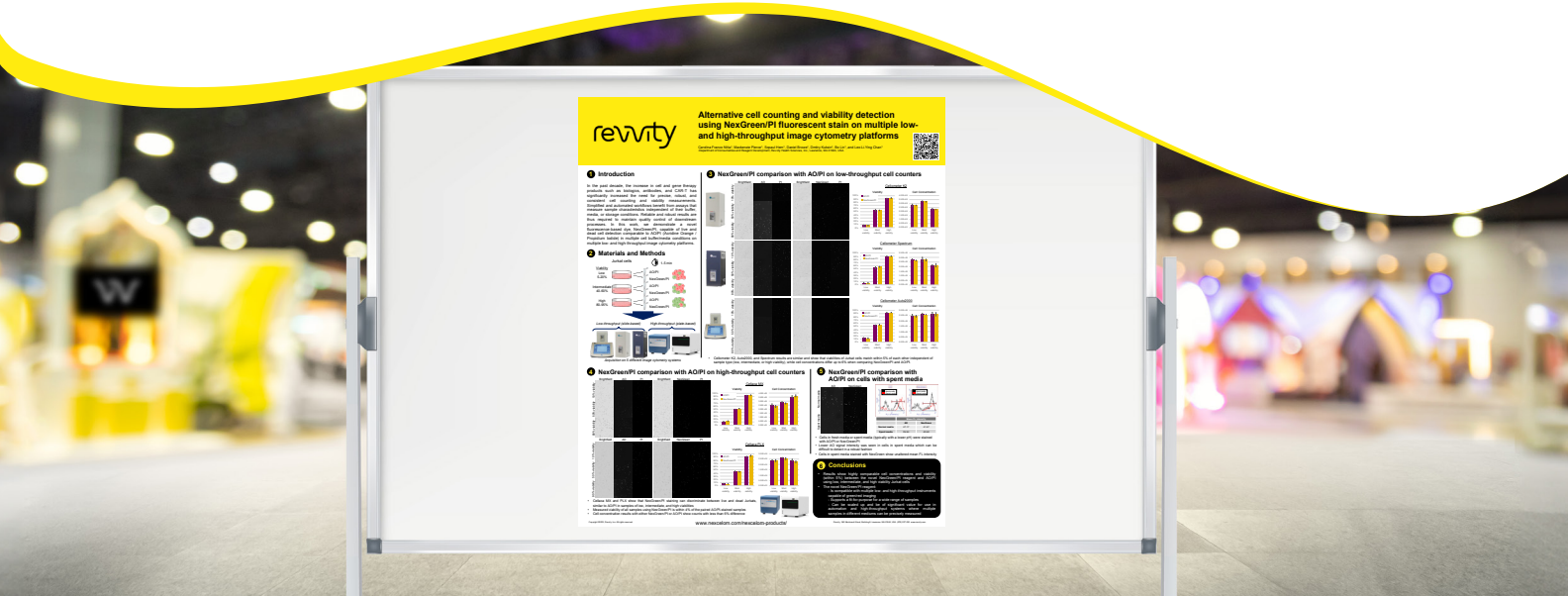
Introduction

In the past decade, the increase in cell and gene therapy products such as biologics, antibodies, and CAR-T has significantly increased the need for precise, robust, and consistent cell counting and viability measurements. Simplified and automated workflows benefit from assays that measure sample characteristics independent of their buffer, media, or storage conditions. Reliable and robust results are thus required to maintain quality control of downstream processes. In this work, we demonstrate a fluorescence-based dye, NexGreen/PI, capable of live and dead cell detection comparable to AO/PI (Acridine Orange / Propidium Iodide) in multiple cell buffer/media conditions on multiple low- and high-throughput image cytometry platforms.

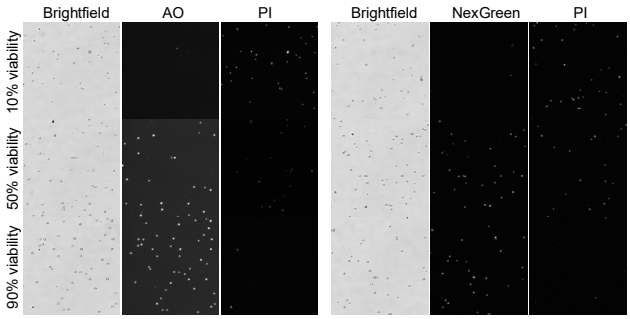
Materials and Methods

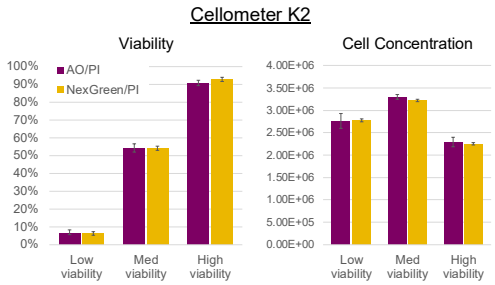


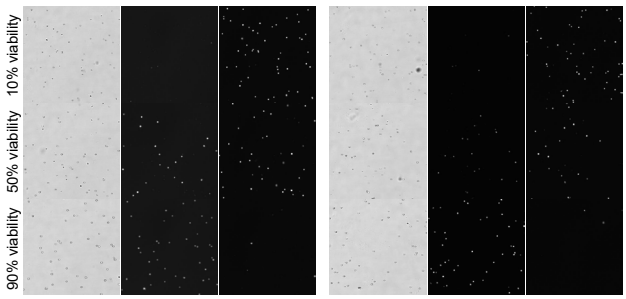
Acquisition on 5 different image cytometry systems

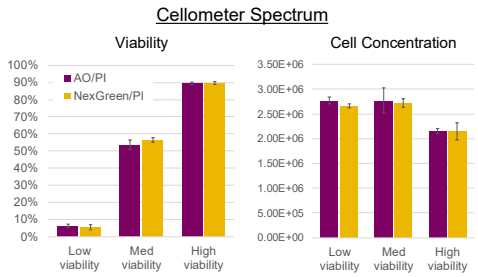


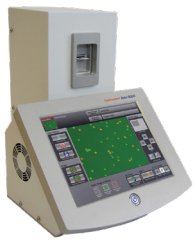
NexGreen/PI stain comparison with AO/PI on low-throughput cell counters

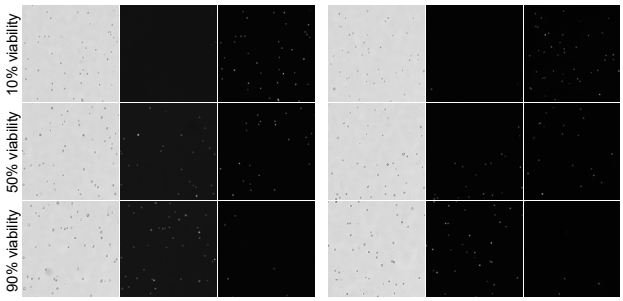


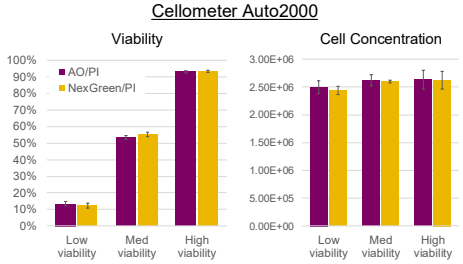






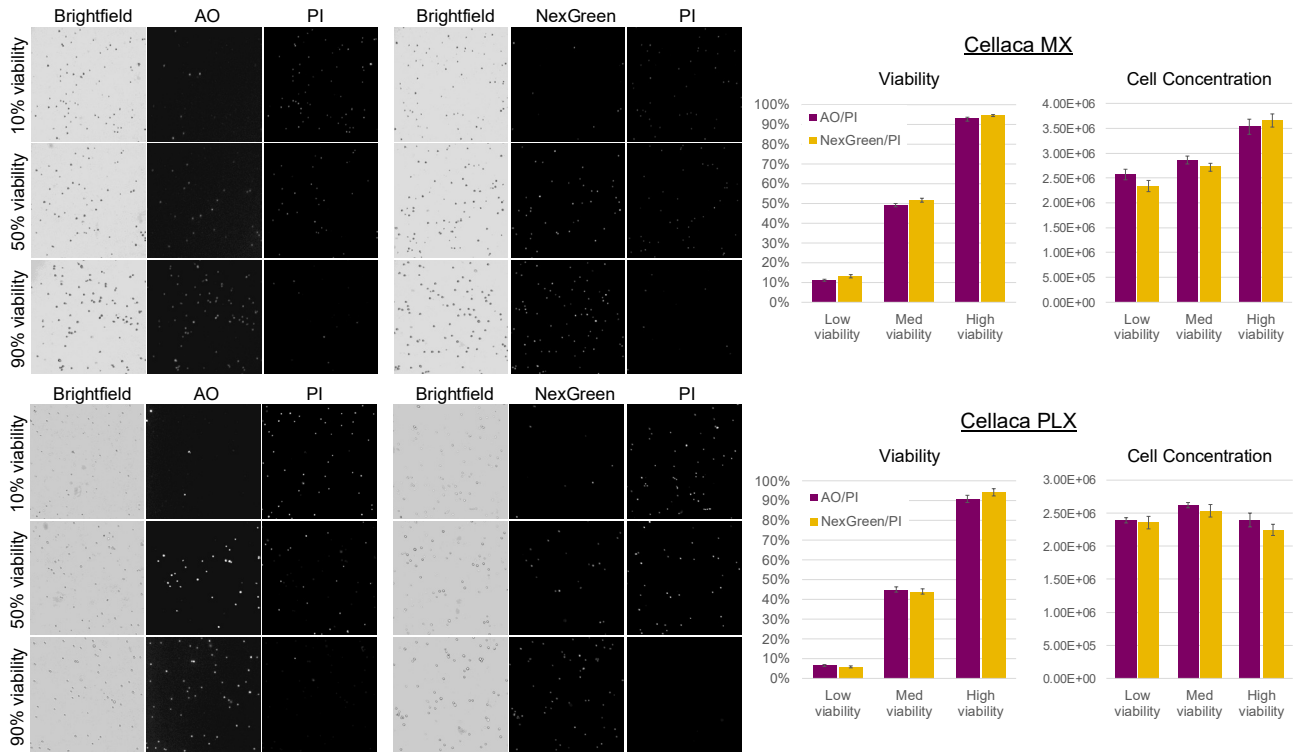




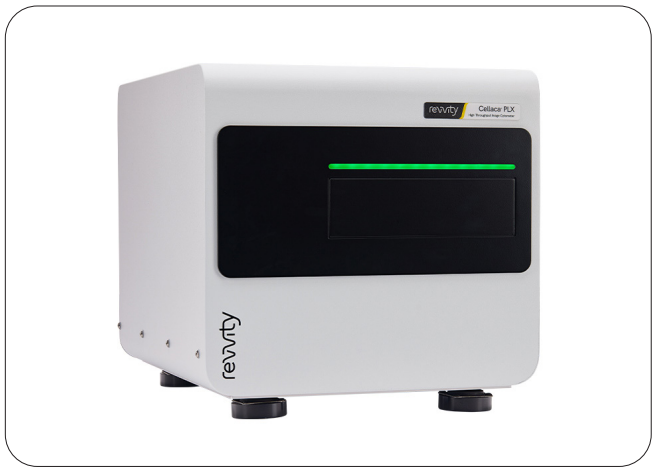


- Cellometer™ K2, Auto2000™, and Spectrum™ systems results are similar and show that viabilities of Jurkat cells match within 5% of each other independent of sample type (low, intermediate, or high viability), while cell concentrations differ up to 6% when comparing NexGreen/PI and AO/PI.

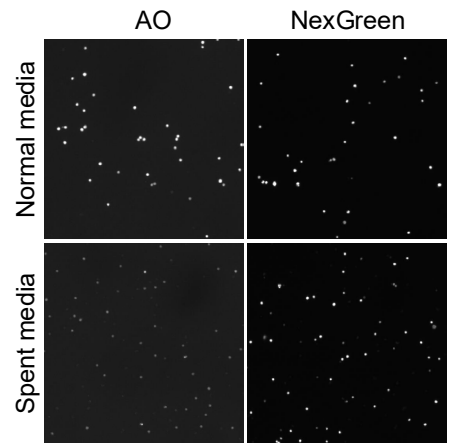
NexGreen/PI stain comparison with AO/PI on high-throughput cell counters

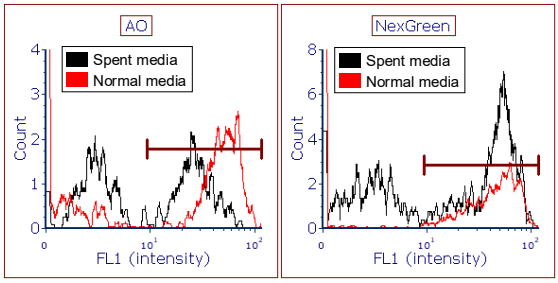


- Cellaca™ MX and PLX platforms show that NexGreen/PI staining can discriminate between live and dead Jurkats, similar to AO/PI in samples of low, intermediate, and high viabilities
- Measured viability of all samples using NexGreen/PI stain is within 4% of the paired AO/PI-stained samples
- Cell concentration results with either NexGreen/PI or AO/PI stains show counts with less than 6% difference



NexGreen/PI stain comparison with AO/PI on cells with spent media





	Mean FL intensity	
	AO	**NexGreen**
Normal media	47.17	41.57
Spent media	25.94	40.50

- Cells in fresh media or spent media (typically with a lower pH) were stained with AO/PI or NexGreen/PI
- Lower AO signal intensity was seen in cells in spent media which can be difficult to detect in a robust fashion
- Cells in spent media stained with NexGreen show unaltered mean FL intensity

Conclusions

- Results show highly comparable cell concentrations and viability (within 5%) between the novel NexGreen/PI reagent and AO/PI using low, intermediate, and high viability Jurkat cells
- The NexGreen/PI reagent:
  - Is compatible with multiple low- and high-throughput instruments capable of green/red imaging
  - Supports a fit-for-purpose for a wide range of samples
  - Can be scaled up and potentially be of significant value for use in automation and high-throughput systems where multiple samples in different mediums can be precisely measured