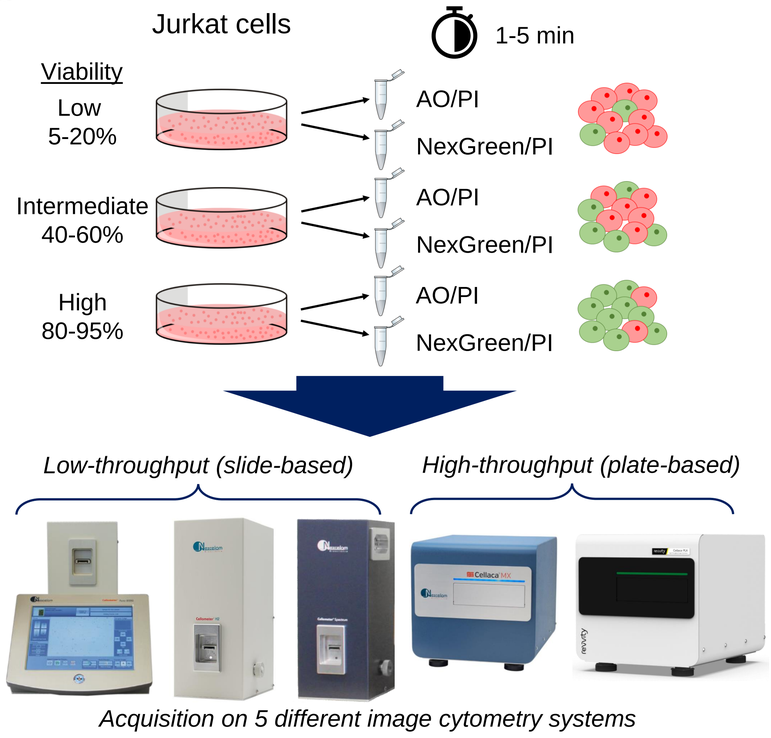
Alternative cell counting and viability detection using NexGreen/PI fluorescent stain on multiple low- and high-throughput image cytometry platforms

Carolina Franco Nitta[1], Mackenzie Pierce[1], Sopaul Hem[1], Daniel Browe[1], Dmitry Kuksin[1], Bo Lin[1], and Leo Li-Ying Chan[1]
[1]Department of Consumables and Reagent Development, Revvity Health Sciences, Inc., Lawrence, MA 01843, USA

1 Introduction

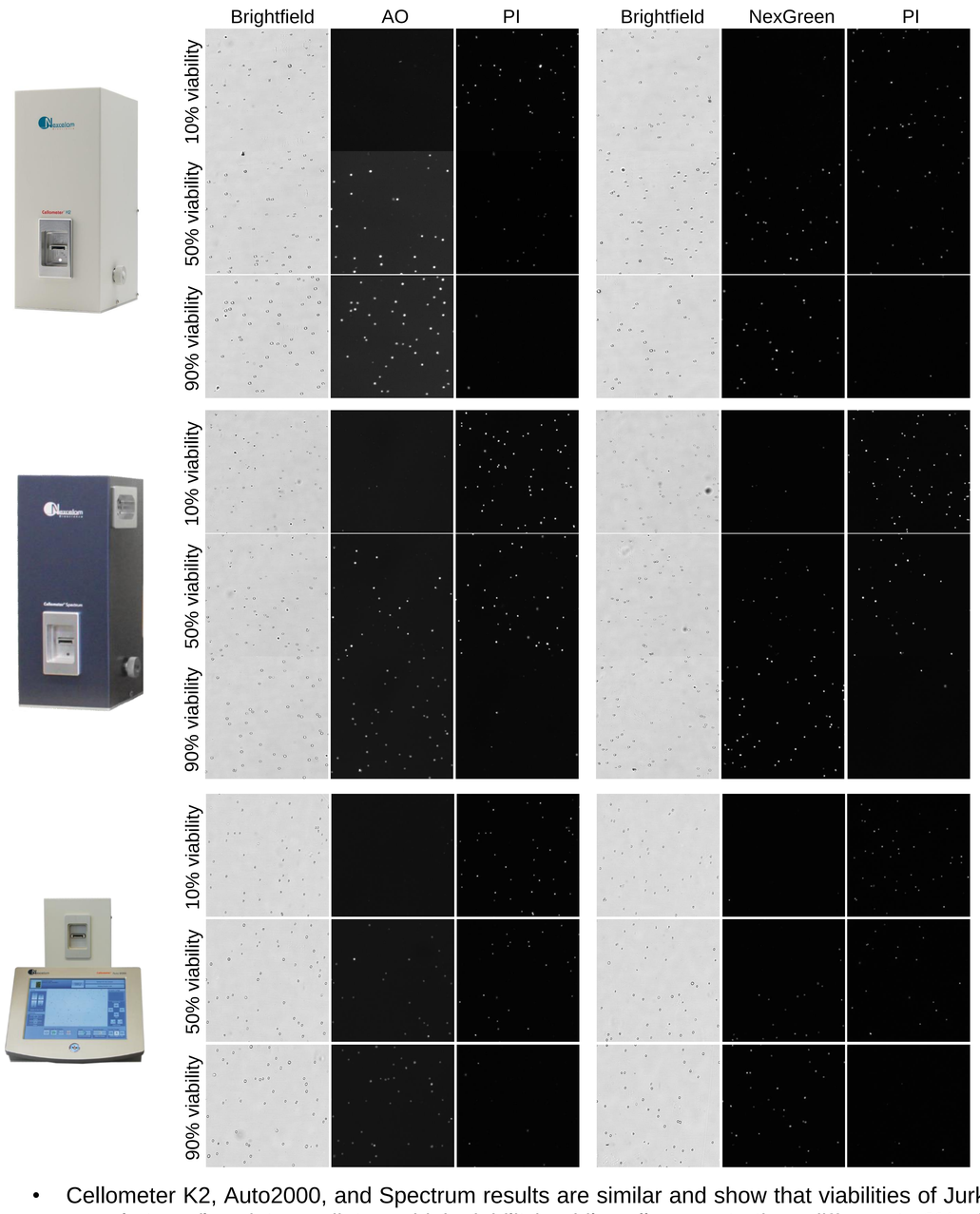
In the past decade, the increase in cell and gene therapy products such as biologics, antibodies, and CAR-T has significantly increased the need for precise, robust, and consistent cell counting and viability measurements. Simplified and automated workflows benefit from assays that measure sample characteristics 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 novel 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.

2 Materials and Methods



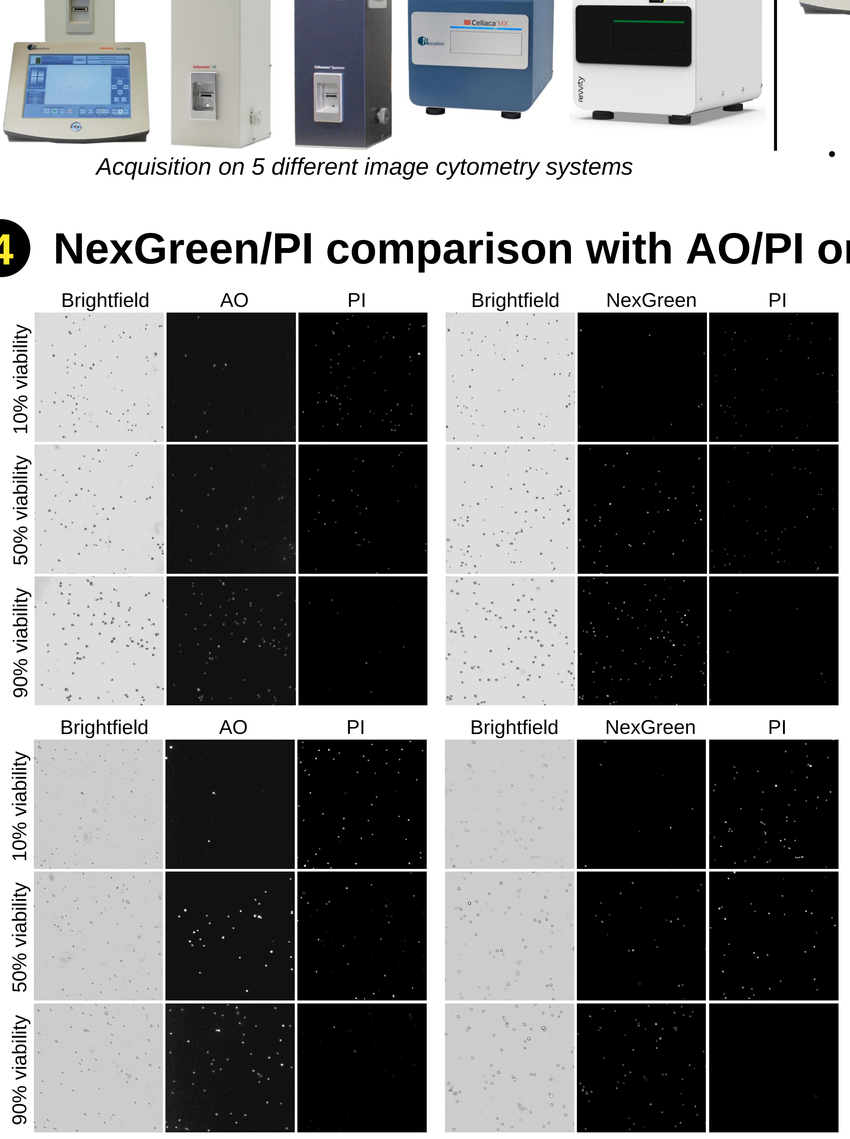
Acquisition on 5 different image cytometry systems

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



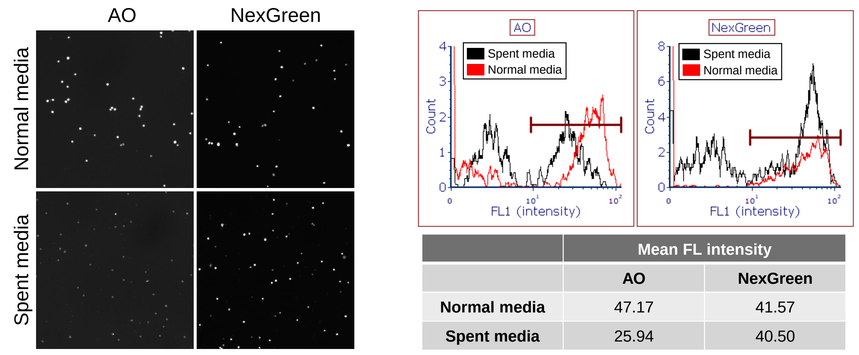
- Cellometer K2, Auto2000, and Spectrum results are similar and show that viabilities of Jurkat cells match within 5% of each other independent of sample type (low, intermediate, or high viability), while cell concentrations differ up to 6% when comparing NexGreen/PI and AO/PI.

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



- Cellaca MX and PLX 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 is within 4% of the paired AO/PI-stained samples
- Cell concentration results with either NexGreen/PI or AO/PI show counts with less than 6% difference

5 NexGreen/PI 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

6 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 novel 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 be of significant value for use in automation and high-throughput systems where multiple samples in different mediums can be precisely measured

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Revvity, 360 Merrimack Street, Builiding 9, Lawrence, MA 01843, USA (978) 327-530 www.revvity.com