Cellometer Ascend: Cell count, viability, and cell-based assays.

The Cellometer Ascend is Revvity's next generation cell counter designed to produce reliable cell counting results for various biological research fields, such as cell and gene therapy, regenerative medicine, bioprocessing, immuno-oncology, and virology/infectious disease.

Earlier generations of the Cellometer platform have demonstrated reliable cell counting following the ISO Cell Counting Standards part 1 and 2 (1, 2). The Cellometer Ascend further improves the quality of cell counting results through the introduction of three advanced technologies:

1. Acquisition of a larger volume of cells for analysis per sample, which may improve cell counting precision while reducing image acquisition time.
2. Novel slide-sensing technology and software features to automate the cell counting workflow further improving the cell counting efficiency.
3. A proprietary autofocusing technology for efficient segmentation and analysis of samples, which enables counting of low cell concentration (below 1 x 10^5 cells/mL).



Materials and methods

Cell lines and whole blood

Jurkat cells (Clone 36-1; ATCC, cat # TIB-152) were cultured in RPMI 1640 media (Thermo Fisher; cat # 11875-119) supplemented with 10% FBS (Access Biologicals, US Origin) and 100 U/mL Pen/Strep (Thermo Fisher, cat # 15140-122). FreeStyle™ CHO-S cells (Thermo Fisher, cat # R80007) were cultured in FreeStyle™ CHO Expression media (Thermo Fisher Scientific, cat # 12651-022), supplemented with 8 mM L-glutamine (ATCC, cat # 30-2214). K-562-GFP cells (ATCC, cat # CCL-243-GFP) were cultured in Complete McCoy's 5A (Sigma, cat # M9309-500ML) modified media supplemented with 10% FBS and 100 U/mL Pen/Strep. HepG2 cells (ATCC, cat # HB-8065) were cultured in Eagle's Minimum Essential Medium (EMEM; ATCC, cat # 30-2003) supplemented with 10% FBS and 100 U/mL Pen/Strep. U2OS-RFP-TUBA1B cells (Sigma, cat # CLL1034-1VL) were cultured using IMDM with L-glutamine media (VWR, cat # 112-035-101) supplemented with 10% FBS, 100 U/mL Pen/Strep, and 0.5 µg/mL puromycin (Thermo Fisher, cat # A1113803). PC-3 cells (ATCC, cat # CRL-1435) were cultured in F-12K media supplemented with 10% FBS and 100 U/mL Pen/Strep. CHO cells were incubated rocking at 37 °C with 8% CO_2 levels, while all other cell lines were incubated at 37 °C with 5% CO_2 levels. Cell concentrations and viability were routinely verified using AO/PI (Revvity, cat # CS2-0106-25mL) on a Cellometer Spectrum (Revvity).

K-562-GFP cells were mixed 1:1 with Jurkats cells to mimic a 50% GFP positive population.

Whole blood was freshly isolated and immediately diluted 1:20 with 1x PBS prior to fluorescent staining with AO/PI.

Viability and vitality cell staining

AO/PI stain (Revvity, cat # CS2-0106-25mL) was mixed 1:1 with cells or nuclei for fluorescent verification of viability and concentration. Trypan Blue (TB, 0.4%; Gibco, cat # 15250-061) was diluted to 0.2% concentration and mixed 1:1 with cells (for a final TB concentration of 0.1%) for viability and concentration measurements. Stained cells (AO/PI or TB) were loaded into a 3-chamber (Revvity, cat # ASD-CHM3-001) or 8-chamber counting slide (ASD-CHM8-001) for image acquisition and analysis on the Cellometer Ascend.

Jurkat cells were placed at 4 °C for 48h to create an unhealthy population. Staining with Calcein AM/PI (Revvity, cat # CSK-0118) was performed according to manufacturer recommendations. Briefly, healthy and unhealthy Jurkat cells (~1 x 10^5 cells in 40 µL) each were stained with 5 µL PI and 5 µL of Calcein AM working solution for 20 min at 37 °C in the dark. Stained cells were loaded into an 8-chamber counting slide for image acquisition and analysis on the Cellometer Ascend.

Nuclei isolation

Nuclei were isolated from Jurkat cells using the Nuclei EZ Prep Kit (Sigma, cat # NUC101). Briefly, 2.5 x 10^7 Jurkat cells with a viability higher than 90% were centrifuged and washed 2 times with ice-cold 1X PBS. Cells were lysed twice with 4 mL of ice-cold Nuclei EZ lysis buffer for 5 min and centrifuged. Supernatant was discarded and isolated nuclei were resuspended and kept in 500 µL of ice-cold Nuclei EZ storage buffer prior to staining.

Nuclear isolation of flash-frozen mouse kidney and liver tissues was performed using the Chromium Nuclei Isolation Kit (10X Genomics, cat # PN-1000494) following manufacturer recommendations. Briefly, kidney and liver samples were placed in dissociation tubes, on ice, 20 µL of lysis buffer was added, and tissues were grinded. Additional 300 µL lysis buffer was added and samples mixed and incubated at 4 °C for 10 min. Dissociated tissues were transferred to a nuclei isolation column/collection tube and centrifuged at 3,000 x rcf for 60 sec at 4 °C. The isolation column was discarded and remaining collected nuclei were vortexed and centrifuged at 500 x rcf for 3 min at 4 °C. The supernatant was removed without disturbing the nuclei pellet. Nuclei were resuspended in 500 µL of debris removal buffer and centrifuged at 700 x rcf for 10 min at 4 °C and supernatant removed. Nuclei pellets were washed 2 times with 1 mL of wash and resuspension buffer each time followed by centrifugation at 500 x rcf for 5 min at 4 °C. Isolated nuclei were finally resuspended in 200 µL of wash and resuspension buffer and kept on ice until staining was performed.

Isolated nuclei from Jurkat cells or mouse tissue were stained with AO/PI and loaded into 3-chamber counting slides and acquired using 8 images per chamber to account for potential low nuclei concentration.

Example cell count and cell-based assays

The Cellometer Ascend performs assays with improved precision and efficiency compared to earlier Cellometer models. Examples of assays commonly performed on the Cellometer Ascend include:

1. **Viability assays -** Used to determine cell viability of a wide variety of samples ranging from cell lines to primary cells for cell and gene therapy and bioprocessing using cellular stains/dyes:
    a. AO/PI: Acridine Orange (AO) and Propidium Iodide (PI)
        i. AO is a nuclear dye that passively penetrates all cells and binds to the DNA, while PI only enters the membrane-compromised dead cells.
        ii. PI quenches the AO fluorescent signal through Fluorescence Resonance Energy Transfer (FRET).
        iii. Live cells with intact membranes will fluoresce green (AO only), whereas dead cells with compromised membranes (containing AO and PI) will fluoresce red due to FRET.
    b. Trypan blue
        i. Trypan blue only enters membrane-compromised dead cells.
        ii. Viability is calculated through brightfield imaging and direct counting of live cells that have a bright center (no dye) and dead cells with a Trypan blue stained (dark) center.

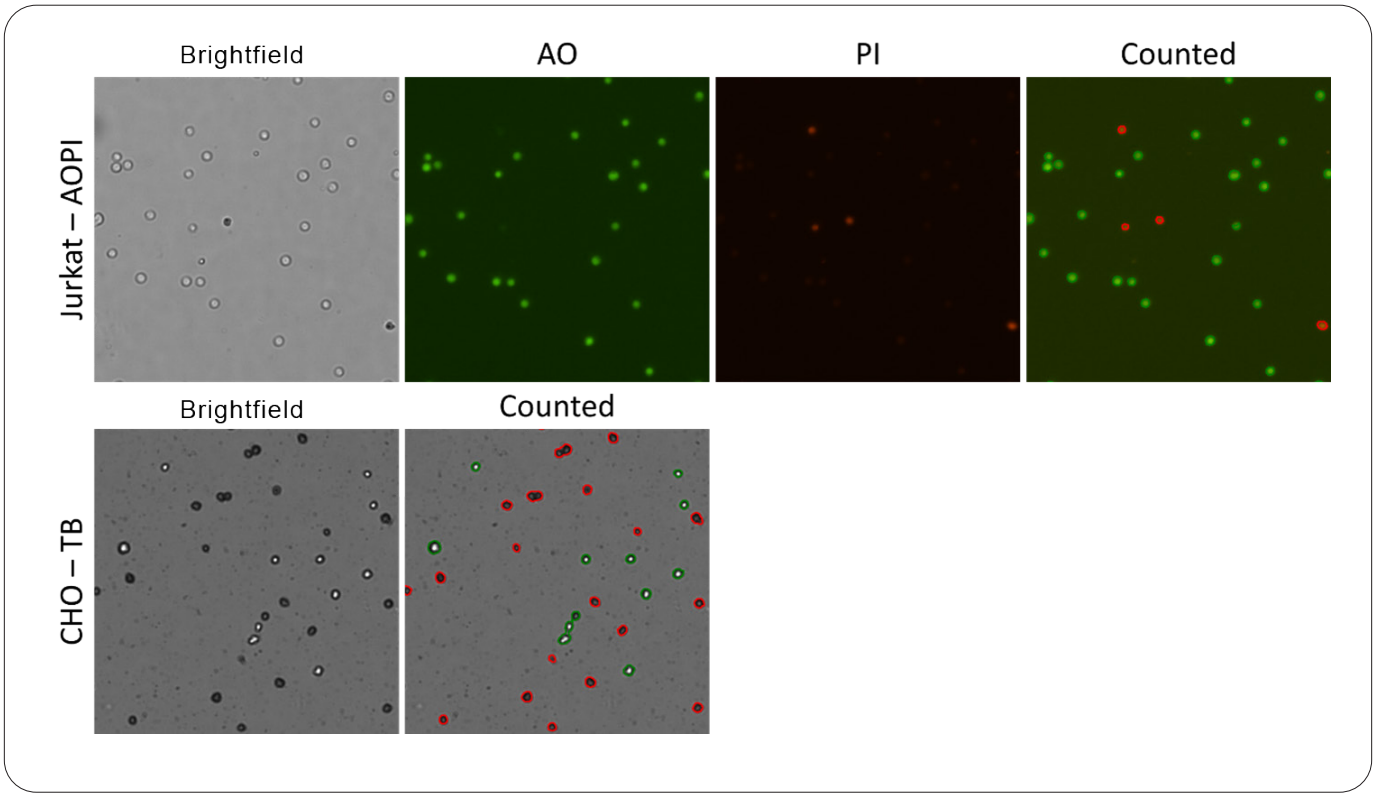


Figure 1: Example cropped brightfield and fluorescent images for viability assays acquired and analyzed using the Cellometer Ascend. The AOPI-stained fluorescent images of Jurkat cells and Trypan blue-stained brightfield images of CHO cells are directly counted to generate cell concentration and viability.

2. Vitality assay - Used to determine cellular enzymatic activity by measuring viable and non-viable cells using fluorescent dyes.

a. Calcein AM and PI

i. Cellular vitality is calculated by Calcein AM staining of metabolically active cells, indicative of healthy behavior, and PI staining of membrane-compromised dead cells.

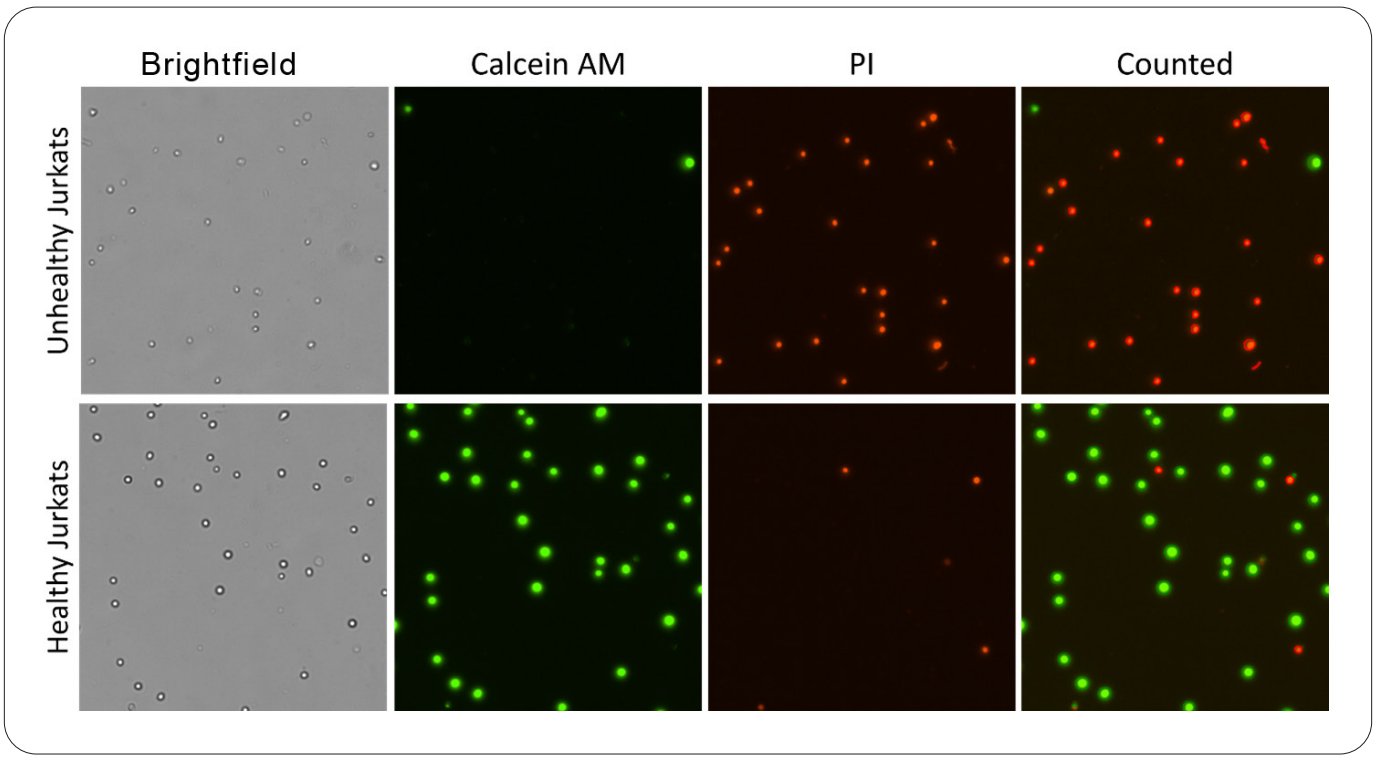


Figure 2: Example cropped brightfield and fluorescent images for vitality assay acquired and analyzed using the Cellometer Ascend. Calcein AM/PI-stained fluorescent images are directly counted to generate cell concentration and vitality. Unhealthy cells (top images) demonstrate low number of cells stained with Calcein AM and high number of cells stained with PI, showing lower vitality. Healthy cells (bottom images) demonstrate the opposite profile, with more cells positively stained for Calcein AM.

3. Nuclei isolation assay - Used after nuclei sample preparation for single-cell sequencing. High quality nuclei preparation and concentration is essential for single-cell analysis.

a. AO/PI on isolated nuclei preparation

i. AO identifies any residual unlysed cells with an intact membrane.

ii. PI can identify all free nuclei and determine the concentration of the free nuclei.

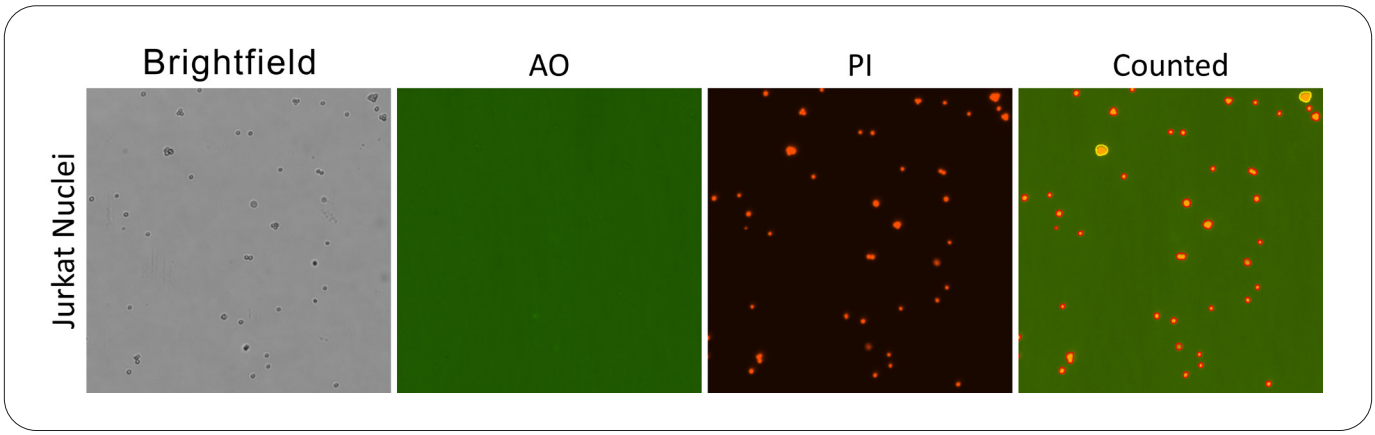


Figure 3: Example cropped brightfield and fluorescent images for isolated nuclei from Jurkat cells stained with AO/PI and acquired and analyzed using the Cellometer Ascend. Fluorescent images show isolated nuclei stained with PI and no residual intact cells in the green channel (AO). The counted PI-stained nuclei will allow for identification of percentage of nuclear clumps (yellow outline) and sample concentration.

4. GFP transfection/transduction efficiency - Widely used in cell and gene therapy, immuno-oncology, and virology assays. It is important to rapidly determine the efficiency of transfection/transduction to move the assay downstream for processes such as flow cytometry analysis or single-cell sequencing.

a. GFP (Green Fluorescent Protein)

i. GFP is widely used as a marker for gene editing, where the efficiency of the editing can be determined by measuring transfection/ transduction efficiency.

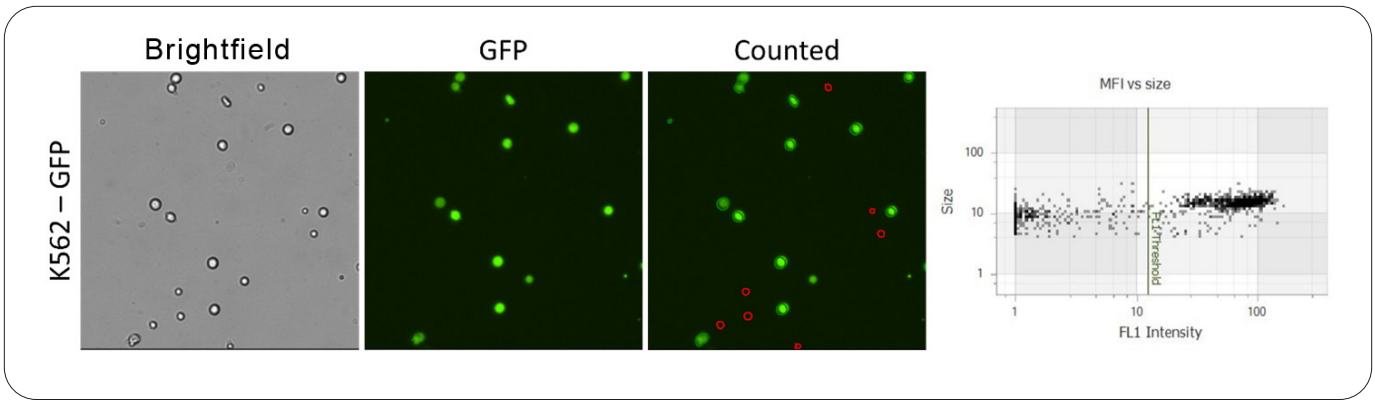


Figure 4: Example cropped brightfield and fluorescent images for K562-GFP cells acquired and analyzed using the Cellometer Ascend. K562-GFP cells were mixed at a 1:1 ratio with non-GFP fluorescing cells for identification of GFP positive and negative cells, as shown in the right-hand scatter plot, and cell concentrations.

Example cell types

The Cellometer Ascend image analysis software (Matrix™) has been used to count a variety of cell types. Some examples are shown below:

1. Cell lines

a. Suspension cells – Cells that grow in an agitated suspension culture individually or as small aggregates.

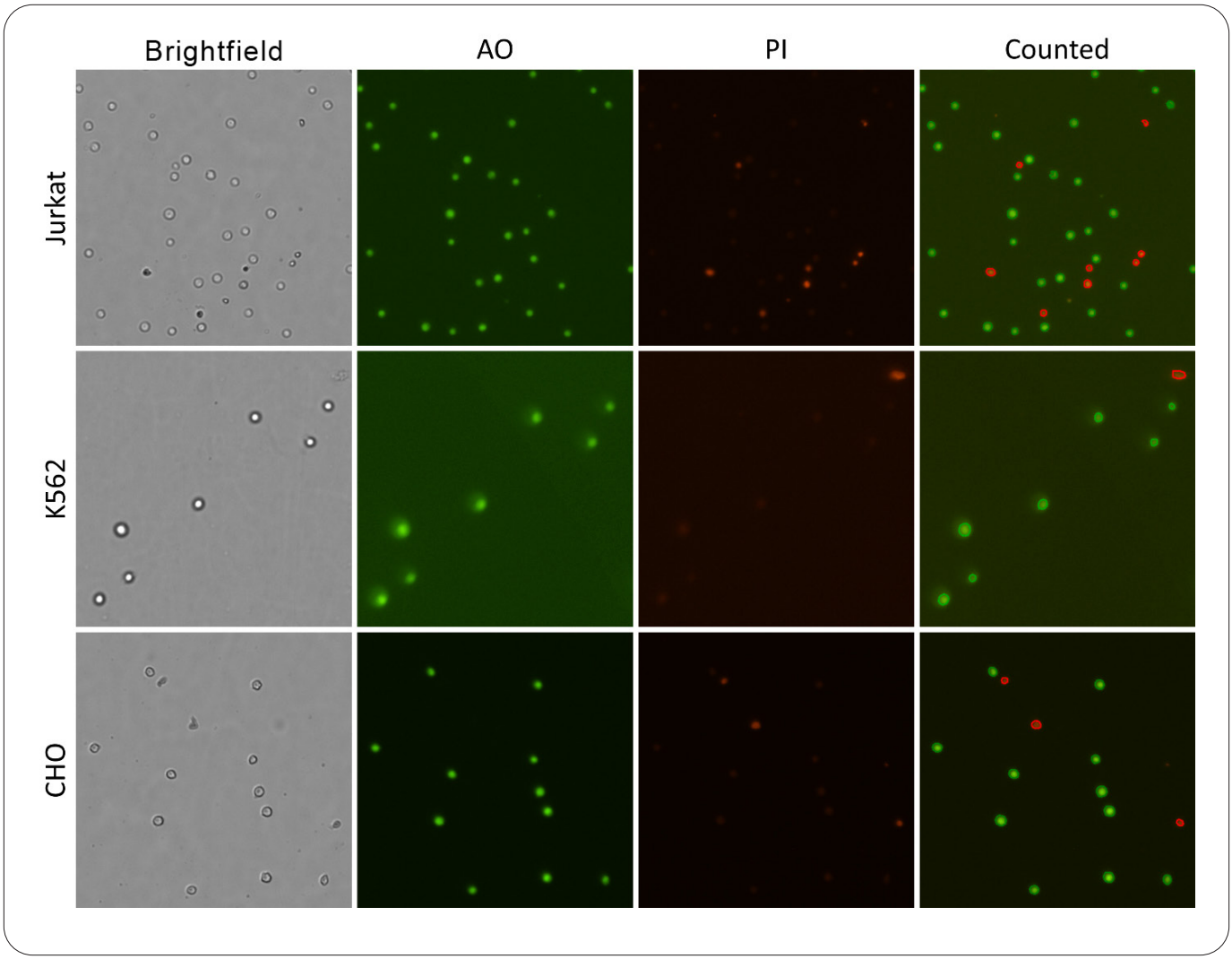


Figure 5: Example cropped brightfield and fluorescent images of AO/PI-stained suspension cell lines Jurkat, K562, and CHO acquired and analyzed using the Cellometer Ascend.

b. Adherent cells – Cells that grow attached to a surface. Most commercially available mammalian cell lines are adherent.

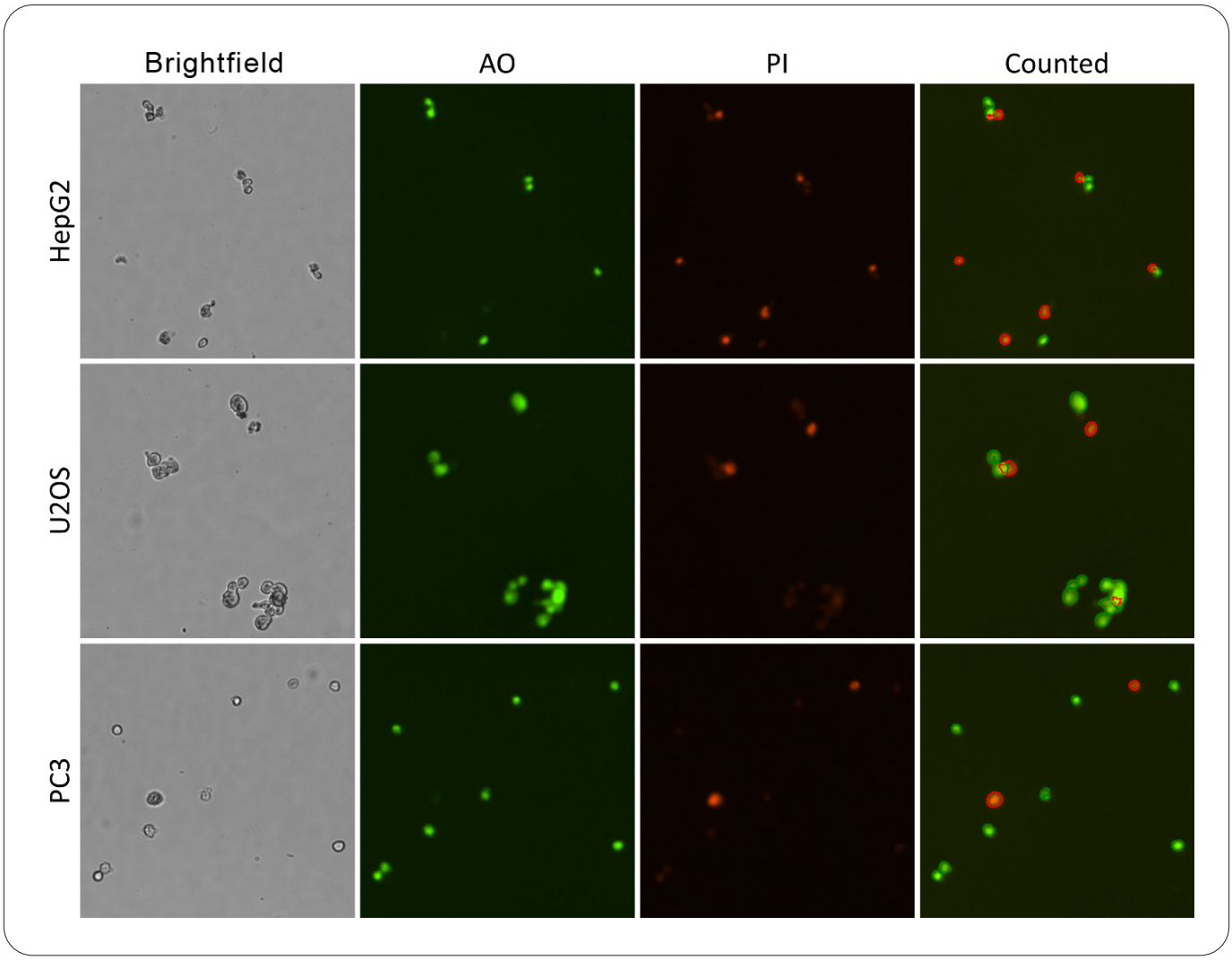


Figure 6: Example cropped brightfield and fluorescent images of AO/PI-stained adherent cell lines HepG2, U2OS, and PC-3 acquired and analyzed using the Cellometer Ascend.

2. Primary cells

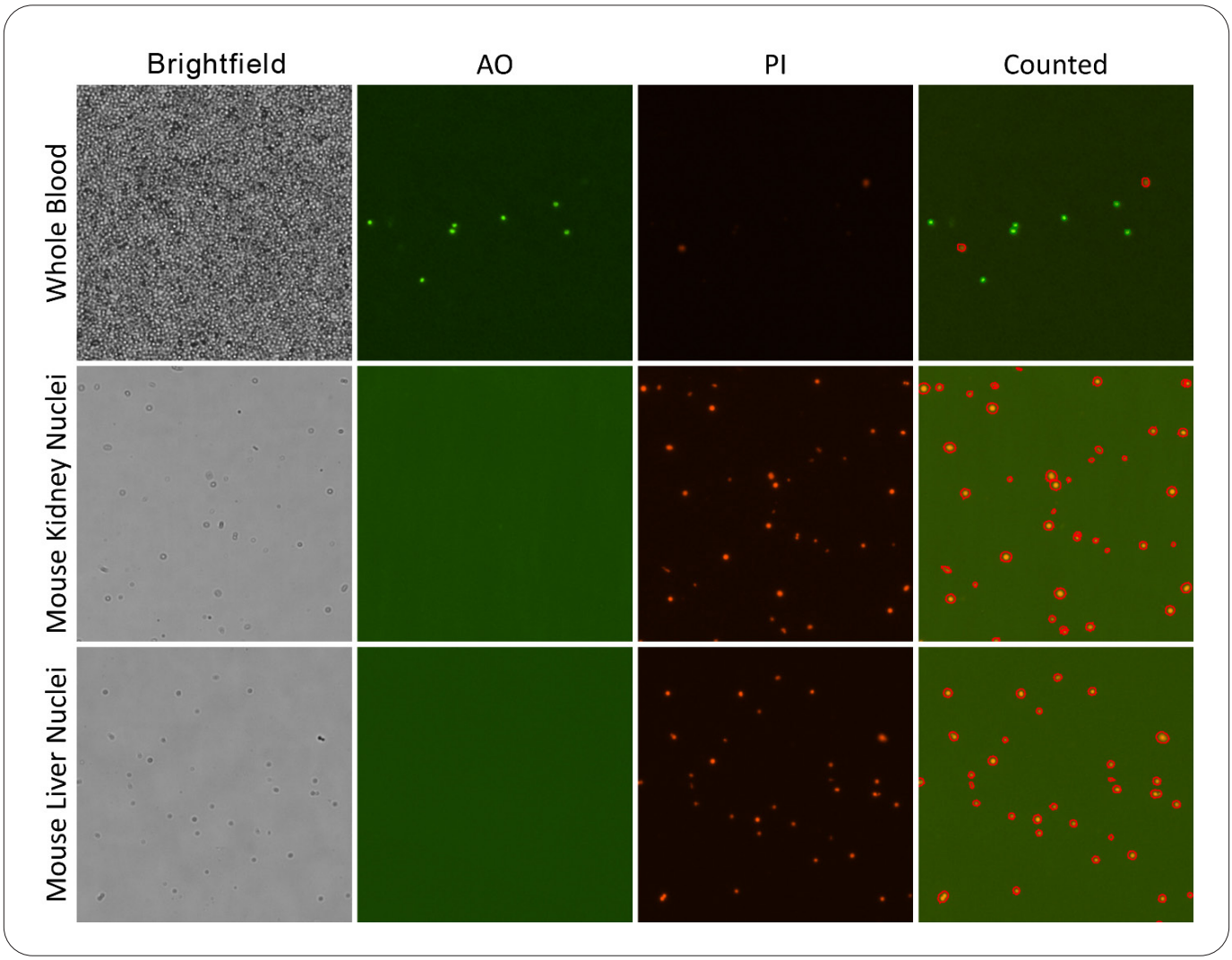


Figure 7: Example cropped brightfield and fluorescent images of AO/PI-stained human whole blood and mouse kidney and liver isolated nuclei acquired and analyzed using the Cellometer Ascend.

Conclusion

Cellometer Ascend has demonstrated flexibility and capability for analyzing numerous cell lines and primary cell types in various cell-based assays such as viability, vitality, GFP transfection/transduction, and nuclei isolation.

1. ISO. Biotechnology — Cell counting — Part 1: General guidance on cell counting methods. International Organization for Standardization 2018;20391-1:2018.
2. ISO. Biotechnology — Cell counting — Part 2: Experimental design and statistical analysis to quantify counting method performance. International Organization for Standardization 2019;20391-2:2019.

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