In Situ Automated Live Cell Confluence and Counting

Tammy M. Joska, PhD, Holden Fried, Kenneth Chapman, Alan Blanchard, PhD
Thrive Bioscience®, Inc., 100 Cummings Center, Suite 306-P, Beverly, MA 01915

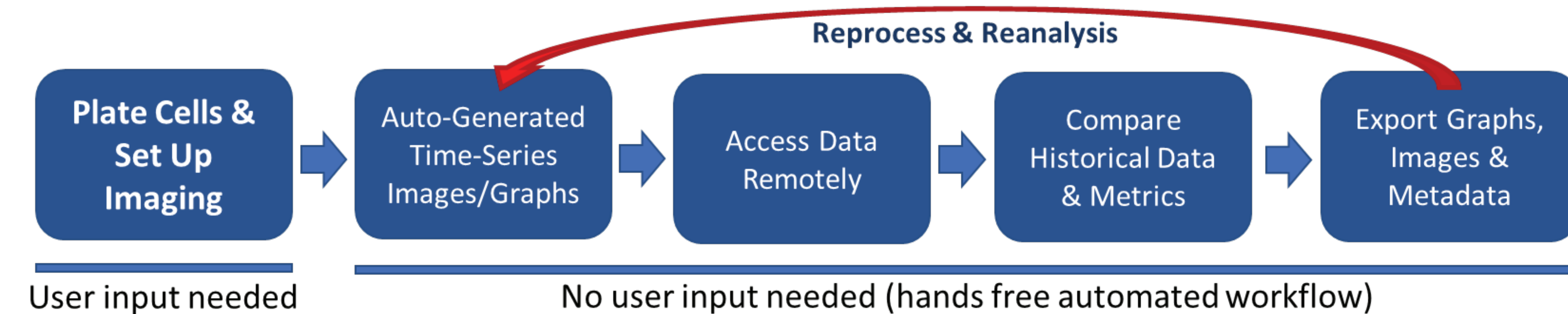
Abstract:

Live cell imaging is a cornerstone in cell biology but manual evaluation of cell health by confluence, doubling rates, and cell counts remain labor intensive and subject to human error. Automated imaging approaches often only provide qualitative data, capture small regions, and frequently require toxic dyes that confound results and harm or kill the cells.

Thrive Bioscience, Inc. uses superior brightfield image stacks to identify live cells and calculate counts and confluence, offering greater reproducibility than manual techniques.

The Thrive CellAssist® system is a tightly integrated hardware and software solution for acquiring, analyzing, storing, and visualizing live cell images. Expandable from a single bench-top unit to a global network of automated, environmentally controlled imagers, it elevates imaging of live cells in culture from an occasional, manual process with sporadic documentation, to a reproducible, routine, automated process that provides unparalleled insight into your cells' behavior.

Confluence and cell counting measurements are validated against hemocytometer readings by comparing Thrive's auto-generated area fraction and cell counting directly from images. In addition, image confluence comparisons were drawn from brightfield image stacks used as input for ImageJ and Thrive's automated co nfluence analysis. *For additional information please visit* www.thrivebio.com.



PROCESS DIAGRAM 1. Live Cell Culture Imaging is Automated Using the CellAssist and CellEval Software. Images are scheduled at a desired frequency and duration. Time-series images and graphs are generated for viewing and analysis in real time or at the conclusion of the experiment. Data can be compared to historical data or re-analyzed with additional data. All data can be exported and saved to a desired location or disc.

PROCESS DIAGRAM 2. Live Cell Culture Imaging with Remote Multi-User Access. Expandable from a single bench-top unit to a global network of automated, environmentally controlled imagers. Data is stored in a centralized location for easy access. Data is available for computer learning, data mining, and artificial intelligence needs.

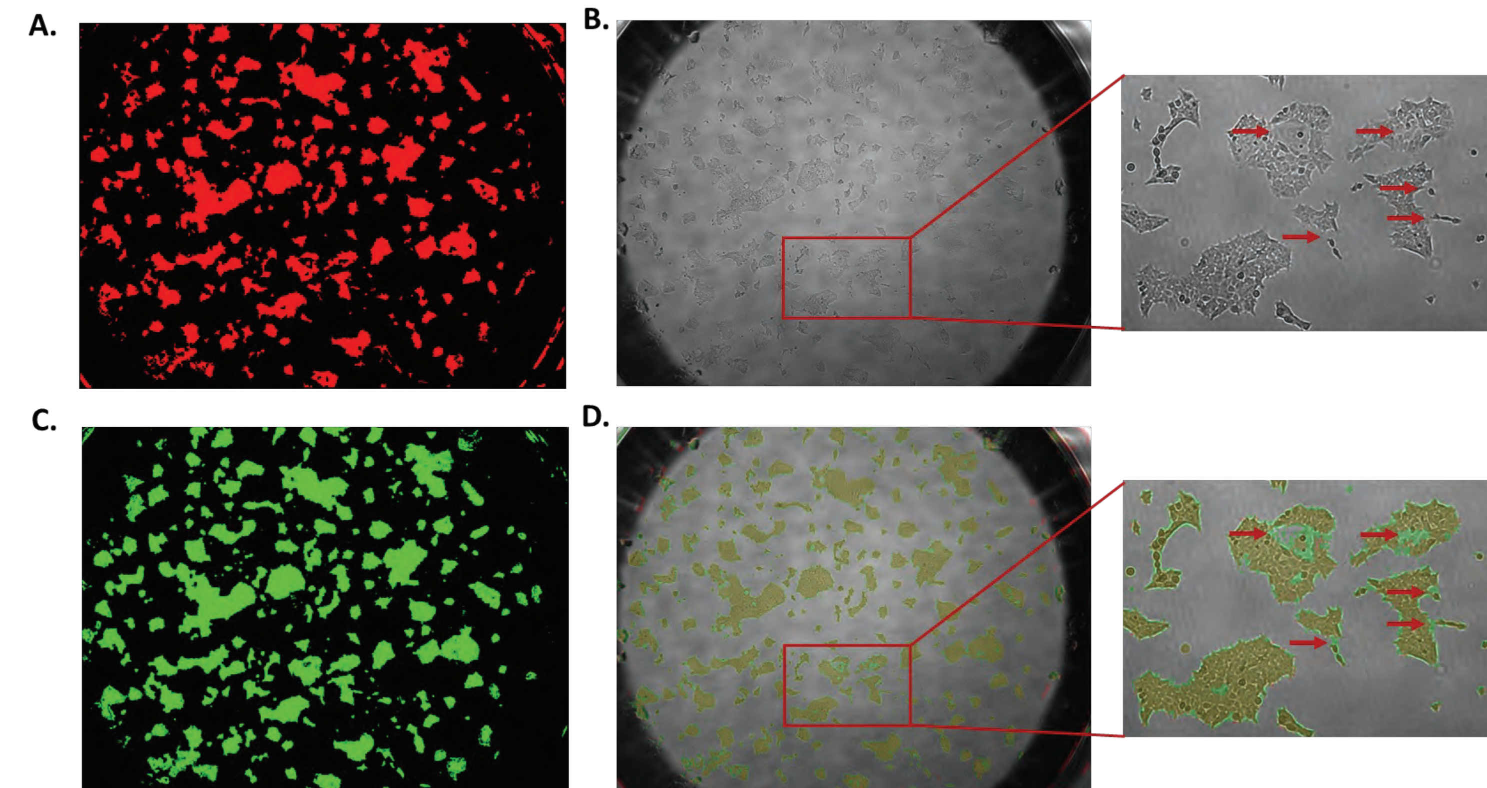


FIGURE 1. Automated Cell Mask Comparisons to an Established Procedure in ImageJ. HEK-293T cells were imaged and analyzed using ImageJ and the Thrive Bioscience CellEval® software package. Panel A represents overlays using the Thrive Bioscience CellEval software package. Panel B, Same image without overlays. Panel C, overlays using ImageJ. Panel D, CellEval overlay (red), superimposed on the ImageJ overlay (green). Red brackets highlight enlarged regions. Arrows signify detection regions that diverge between the two software packages.

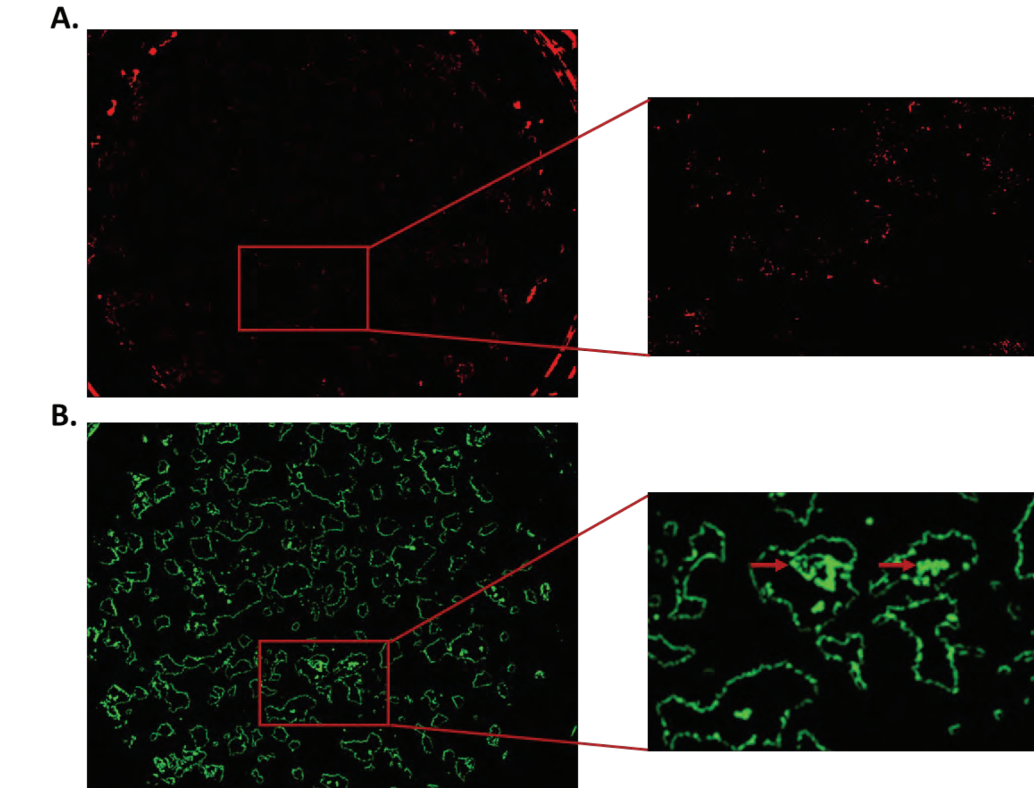


FIGURE 2. Subtracted Masks between Alternate Methods of Confluency Measure. Overlays from CellEval and the ImageJ software packages were subtracted to display the differences in the area detected between the software packages. Panel A, ImageJ outlines with subtracted-out CellEval masks. Panel B, CellEval overlays with ImageJ masks subtracted out. Red brackets show enlarged regions. Arrows signify regions that diverge between the two software packages.

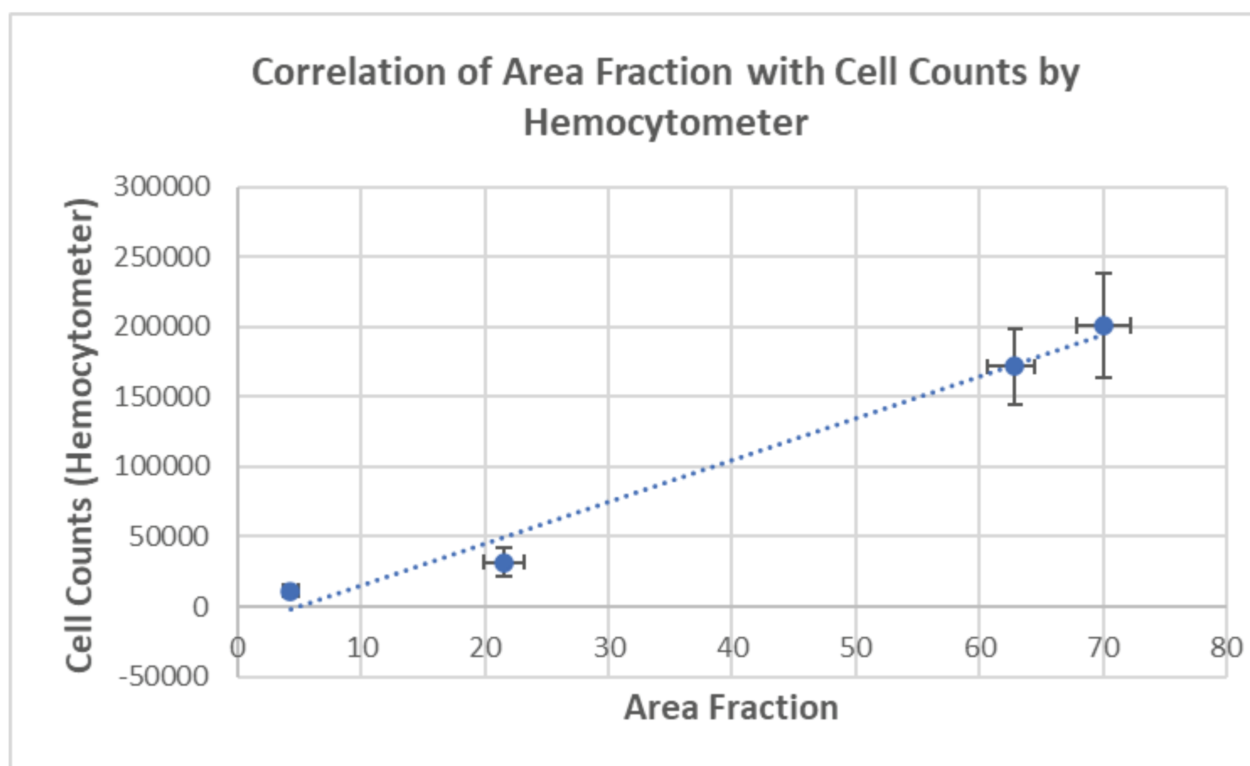


FIGURE 3. Correlation Between Computed Area Fraction and Cell Counts using a Hemocytometer. HEK-293T cells seeded at ~5% confluence were imaged every 4 hours for 4 days. Select time points were chosen to harvest 4 replicates that were counted by hemocytometer (y axis) and compared with respective area fraction calculations (x axis). Averages were plotted with standard deviations. The average correlation co-efficient from Pearson's correlation (n=4) was 0.934 ± 0.05 (p = 0.0085).

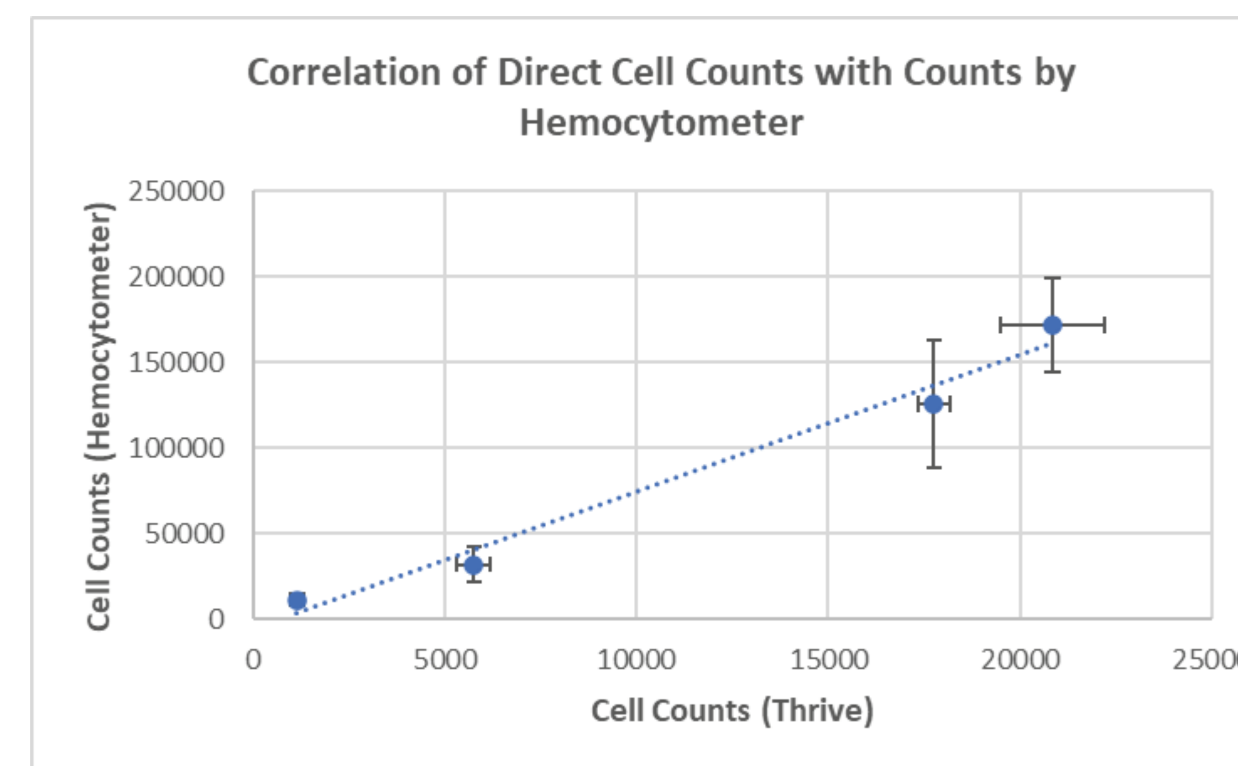


FIGURE 4. Correlation Between Computed Cell Counts and Cell Counts using a Hemocytometer. HEK-293T cells seeded at ~5% confluence were imaged every 4 hours for 4 days. Select time points were chosen to harvest 4 replicates that were counted by hemocytometer (x axis) and compared with respective computed counts (x axis). Averages were plotted with standard deviations. The average correlation co-efficient from Pearson's correlation (n=4) was 0.989 ± 0.05 (p = 0.0043).

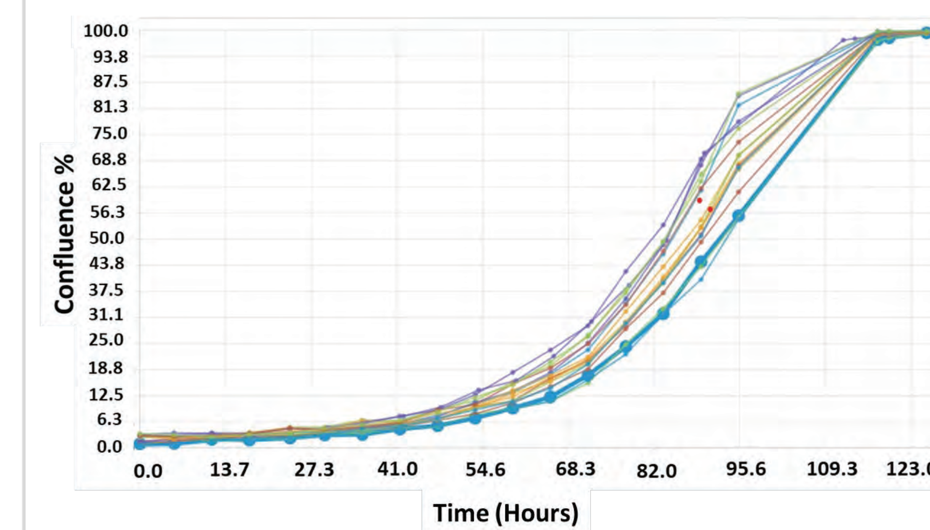


FIGURE 5. Confluence Measures over Time are Auto Generated with all CellAssist Instruments. HEK-293T cells were seeded at ~5% confluence and imaged every 4 hours for ~4 days using brightfield imaging. Each line in the graph represents HEK-293T cell growth over time for each of 24 wells. Y axis- confluence %, X-axis, time in hours.

CONCLUSION:

Measurements of confluence and cell counts were obtained automatically from live cell image stacks without the use of harmful dyes or labels. Computed measurements were highly correlated with manual counting by hemocytometer. Note: counts by hemocytometer include a greater degree of variability compared to automated measures, as expected.

Presented by Thrive Bioscience, Inc. at American Society for Cell Biology, Cell Bio 2022, December 2022, Washington, D.C.

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