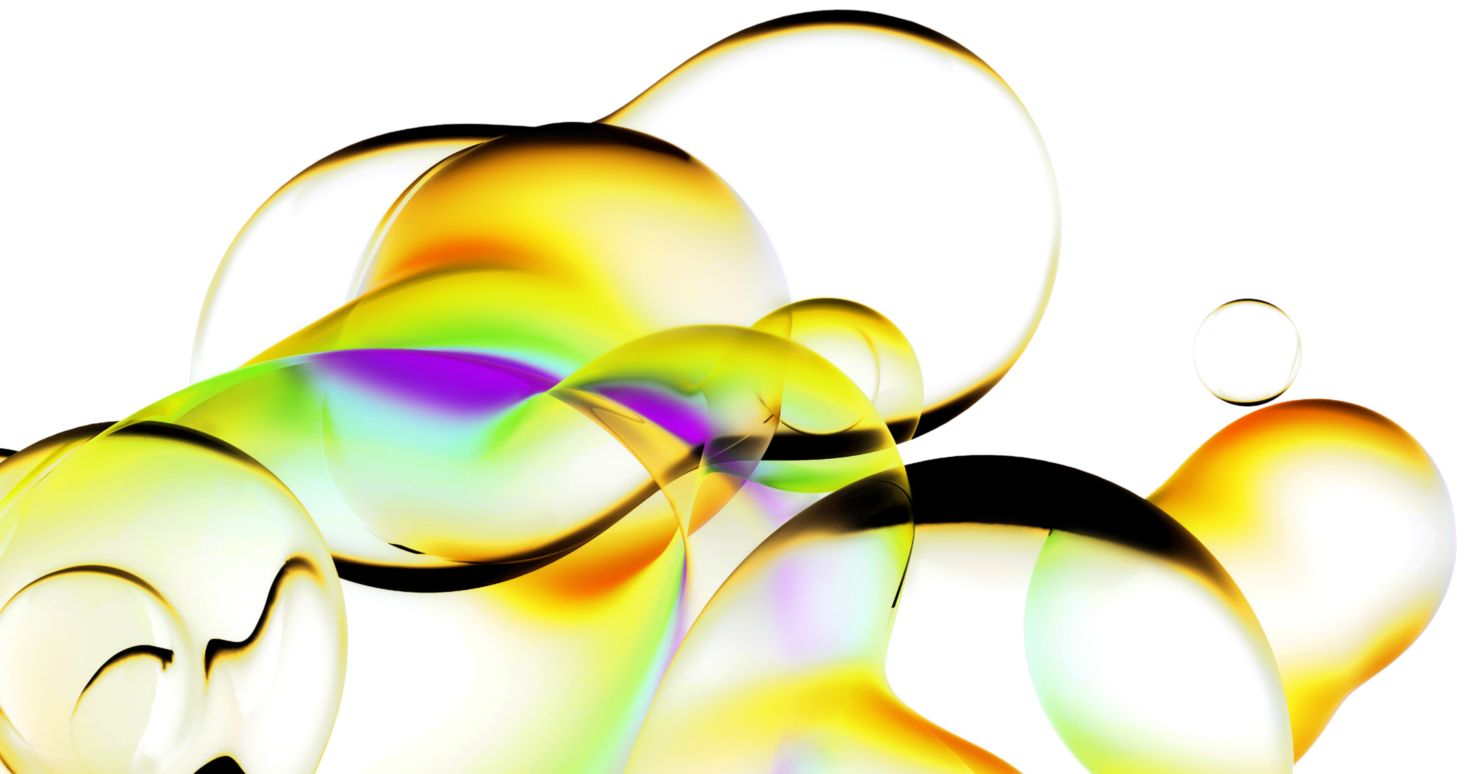
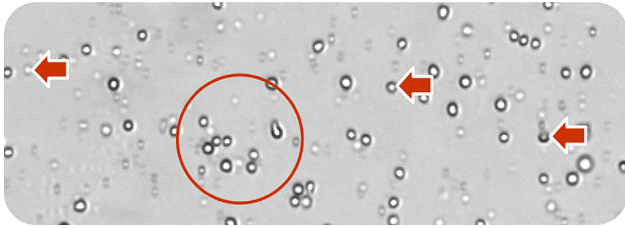
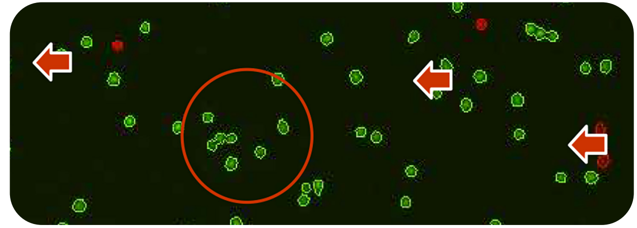
# Cell counting: Are you using the right method?

Fit-for-purpose methodologies are critical to researchers seeking accurate results. Inaccurate results from choosing the wrong cell counting method can lead to consequences such as lower quality of products, rejected publications, repeated experiments, and lost time.

U.S. Congress as well as the National Institute of Standards and Technology (NIST), and other stakeholders, have identified that cell counting and viability measurement assurance are important standards for improving the quality of cell therapy products.



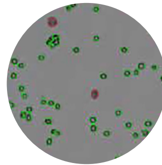




## Brightfield with Trypan blue

- Trypan blue enters cells that have compromised membranes, binding to the intracellular proteins to make them appear blue.
- Dead cells are shown as dark blue, but it may be difficult to distinguish between varying shades of blue to determine if a cell is dead.
- Viable cells do not take up Trypan blue and have clear centers with dark edges.
- Inability to discriminate nucleated cells from non-nucleated cells can lead to overestimation of cell viability in certain cell samples.
- The effect of Trypan blue on certain cell types can lead to overestimation of cell viability due to rupturing of cells.

**When brightfield is a good option**

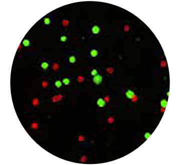


- Clean sample with little debris
- Homogeneous sample
- Working with cell lines

## Fluorescence with AO/PI

- Acridine orange (AO) and propidium iodide (PI) are nuclear staining (nucleic acid binding) dyes.
- AO is permeable to both live and dead cells and stains all nucleated cells to generate green fluorescence.
- PI enters dead cells with compromised membranes and stains all dead nucleated cells to generate red fluorescence.
- Only nucleated cells are stained, debris and RBCs are not stained.
- Provides a clear yes/no answer to generate incredibly accurate viability results even in the presence of clumping, debris, platelets and red blood cells.

**When fluorescence is a good option**



- Messy sample with significant debris
- Heterogeneous sample
- Low concentration of cells

## High-throughput cell counting

The Cellaca™ MX can image, analyze, and report cell concentration and viability for 24 samples in 48 seconds using Trypan blue or 3 minutes using fluorescence.

For more information, visit www.revvity.com

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