

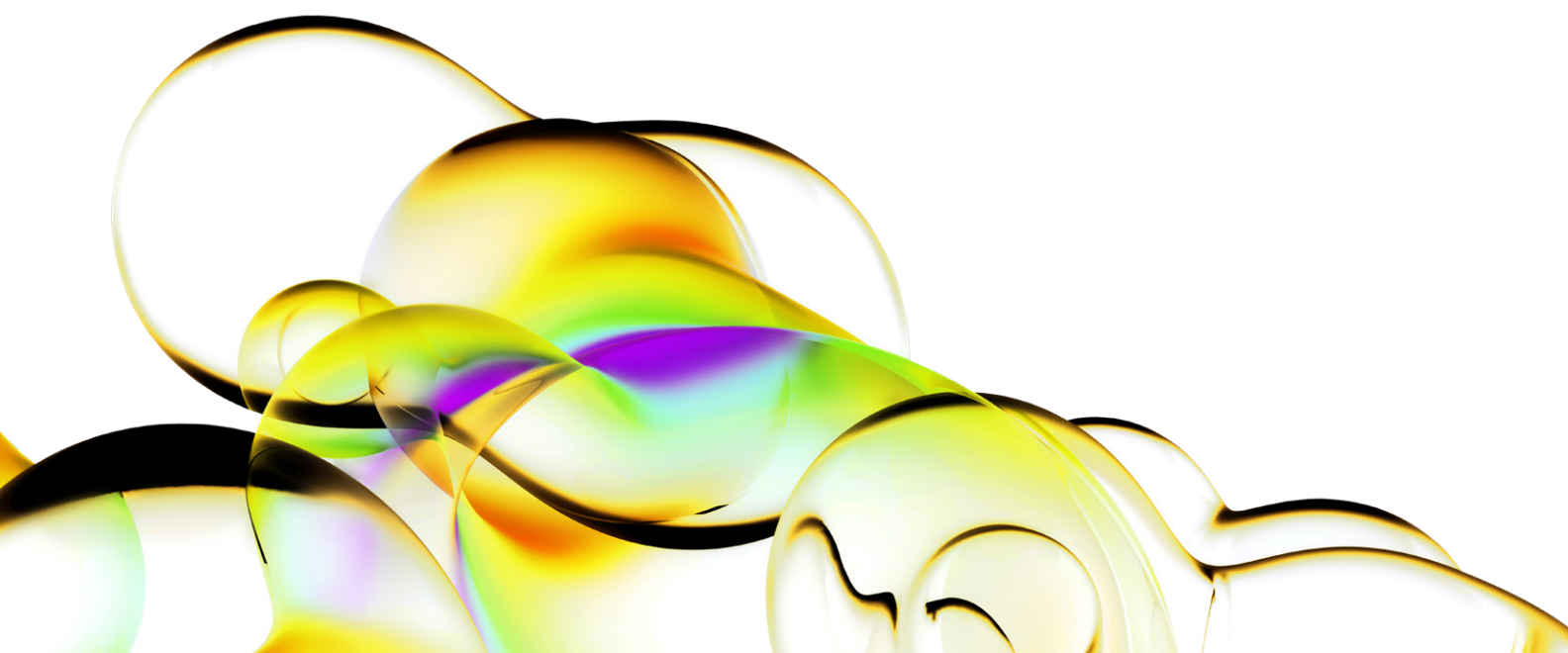
## Cell counting repeatability and consistency.

### Cellometer Auto T4

Cell counting accuracy is governed by two major factors. The first one is the homogeneity of cell suspension when sample is taken for measurement. Typical mammalian cells do not suspend in solution. Without thorough mixing and declumping procedure, sample taken for cell concentration measurement will not reflect cell concentration in the original sample.

When a hemacytometer is used, after the sample is loaded, the operator has to make judgment under a microscope. The variation in judgment from person to person will also affect the cell concentration and viability.

In a Cellometer® Auto T4 cell counter, the software automatically analyzes acquired cell images and measures cell concentration based on imaging analysis using defined judgment parameters. It produces consistent analysis without person to person variation.



## Experiment 1. Measure cell concentration for various cell samples

The following cell counting experiments were performed by different labs. For each cell sample, the same mixing procedure was used prior to taking 20  $\mu$ L for loading a Cellometer counting chamber.

For each cell sample, cell parameters were optimized prior to the counting experiment. N is the total number of sampling for each cell type.

Table 1: Measurement consistency using Cellometer Auto T4

| Cell sample    | Mean concentration  | Std dev             | CV    | N  |
|----------------|---------------------|---------------------|-------|----|
| Mouse CD4 #1   | 2.2x10 <sup>6</sup> | 7.7x10 <sup>4</sup> | 3.5%  | 5  |
| Mouse CD4 #2   | 2.1x10 <sup>6</sup> | 1.3x10 <sup>5</sup> | 6.5%  | 10 |
| Mouse CD4 #3   | 2.1x10 <sup>6</sup> | 1.4x10 <sup>5</sup> | 6.6%  | 14 |
| Mouse B Cell   | 9.7x10 <sup>6</sup> | 7.5x10 <sup>4</sup> | 7.8%  | 7  |
| Zebra fish RBC | 1.7x10 <sup>6</sup> | 1.4x10 <sup>5</sup> | 8.3%  | 7  |
| Jurkat         | 2.5x10 <sup>6</sup> | 2.5x10 <sup>5</sup> | 10.0% | 5  |
| HEPG2          | 1.1x10 <sup>6</sup> | 9.9x10 <sup>5</sup> | 8.9%  | 4  |
| L929           | 1.5x10 <sup>6</sup> | 1.4x10 <sup>5</sup> | 9.4%  | 8  |
| MCF7           | 4.3x10 <sup>5</sup> | 4.2x10 <sup>4</sup> | 9.7%  | 26 |
| MMS            | 1.0x10 <sup>6</sup> | 1.1x10 <sup>5</sup> | 10.3% | 22 |
| HUVEC          | 3.4x10 <sup>5</sup> | 3.3x10 <sup>4</sup> | 9.5%  | 15 |
| 3T3            | 2.1x10 <sup>6</sup> | 2.1x10 <sup>5</sup> | 9.8%  | 6  |
| MDCK           | 1.4x10 <sup>6</sup> | 3.8x10 <sup>4</sup> | 2.8%  | 4  |
| PBMC human     | 2.8x10 <sup>6</sup> | 8.6x10 <sup>4</sup> | 3.1%  | 6  |

## Experiment 2. Repeat measurements of the same cell sample

In this experiment, one 20  $\mu$ L cell or bead samples was loaded into a Cellometer cell counting chamber.

Cellometer Auto T4 cell counter was used to count multiple times, as indicated by N in the follow table.

Table 2: Count the same sample in the same counting chamber repeatedly

| Sample type       | Mean concentration  | Std dev             | CV   | N  |
|-------------------|---------------------|---------------------|------|----|
| Mouse Splenocytes | 3.3x10 <sup>6</sup> | 5.8x10 <sup>4</sup> | 1.7% | 7  |
| 15 $\mu$ m beads  | 7.7x10 <sup>6</sup> | 1.5x10 <sup>4</sup> | 2.0% | 25 |
| Jurkat            | 7.4x10 <sup>5</sup> | 1.1x10 <sup>3</sup> | 0.2% | 3  |
| RCC45             | 3.5x10 <sup>6</sup> | 1.1x10 <sup>4</sup> | 3.2% | 5  |

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