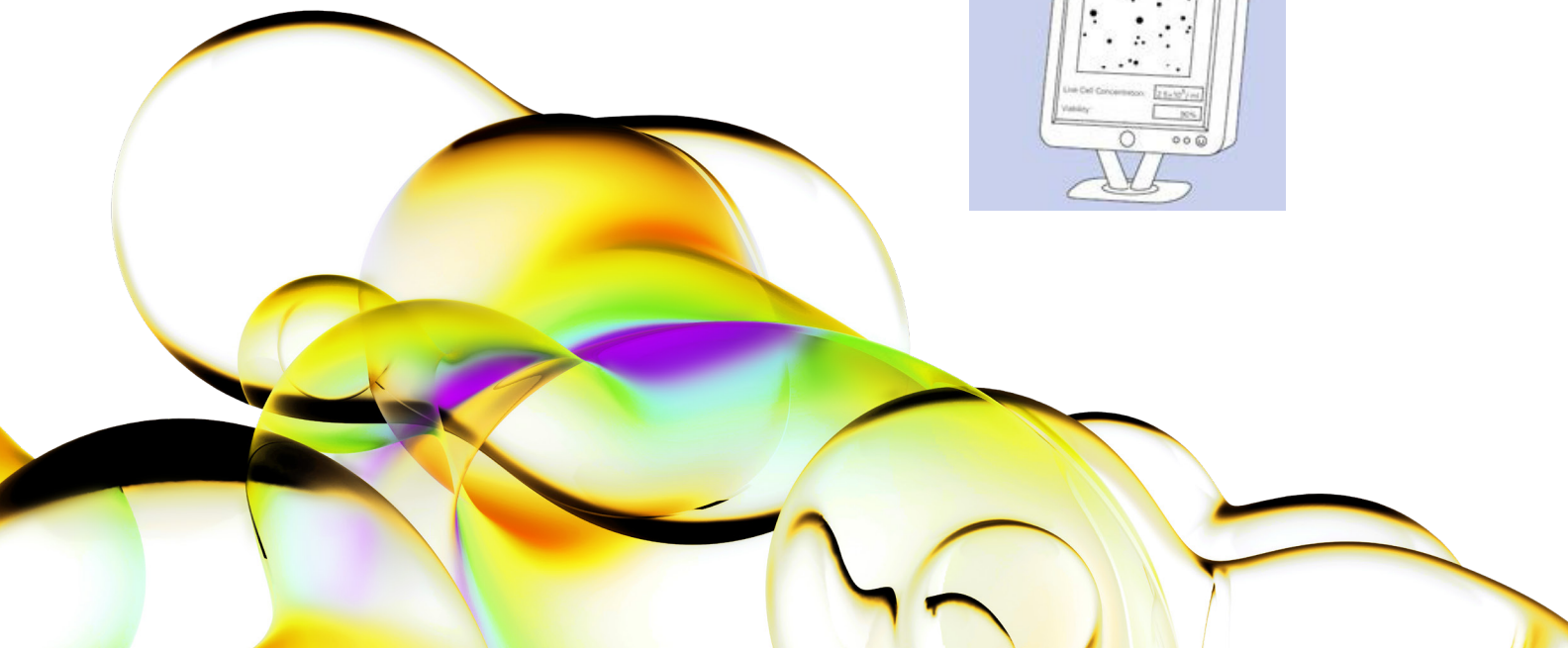
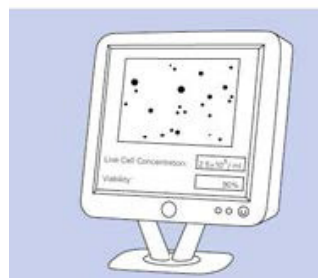
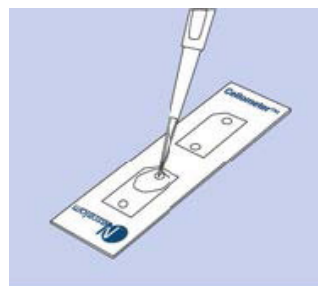


# Insect cell counting and size analysis using Cellometer Auto T4.

## Summary

Insect cells are routinely used in transfection, plaques assays, virus production and protein expression. Cellometer® Auto T4 is used to count cell concentration, viability and also analyze size distribution of an insect cell line SF9. This application note provides some details on method of using the Cellometer system, and shows images and counting data to illustrate a typical application for insect cells. Viability determination is based on trypan blue dye exclusion. Cell concentration is determined automatically based on total cell count and dilution factor.



## Introduction to Cellometer Auto T4

Cellometer Auto T4 is an imaging instrument that acquires cell data from multiple locations of Cellometer disposable counting chambers. It is connected to a computer via USB 2.0 cable. Cellometer Auto T4 software automatically analyzes acquired cell images and measures cell concentration and viability.

There are three simple steps for cell counting with Cellometer Auto T4.

Step 1: Pipette 20  $\mu\text{L}$  cells into Cellometer disposable cell counting chamber.

Step 2: Insert the disposable counting chamber into Cellometer Auto T4 instrument.

Step 3: Inspect cell images; determine concentration and viability automatically.

## Procedure for counting insect cells

1. Mix insect cell culture with trypan blue.
2. Pipette 20  $\mu\text{L}$  of the mixture solution into sample introduction port of a disposable counting chamber (CHT4).
3. Insert chamber slide into Cellometer Auto T4 instrument.
4. Run Cellometer Auto T4 software to obtain cell concentration, viability and cell size diameters.

## Images of insect cells

Figure 1 shows images of SF9 cells. Trypan blue is used for viability measurement.

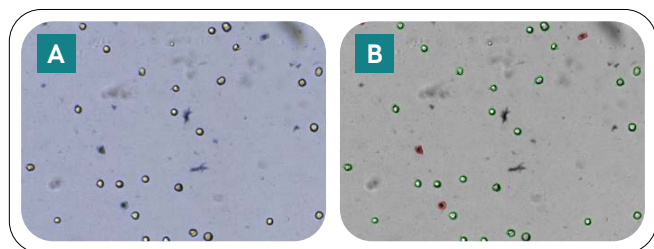


Figure 1: SF9 cell images. Mean cell size equals 18.3  $\mu\text{m}$ . (A) Raw data image and (B) counted cells are indicated by green for live cells and red for dead cells.

Figure 2 shows images from the insect cells that are infected with a virus for producing virus. The sample has very low viability due to the virus production.

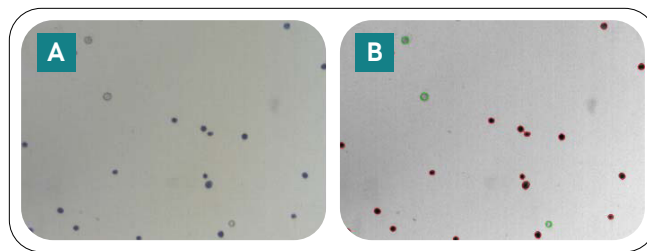


Figure 2: Cellometer Auto T4 measurement results of an infected cell population. (A) Raw image and (B) counted cell image. This cell population has very low viability of 14% after viral infection.

## Cell size analysis

For each cell counting operation, diameter of each counted live cells are saved in a text file. Cell size histogram is presented in "Cell Analysis" function. Figure 3 shows the histogram of a SF9 cells. The mean diameter is 18.3  $\mu\text{m}$ .

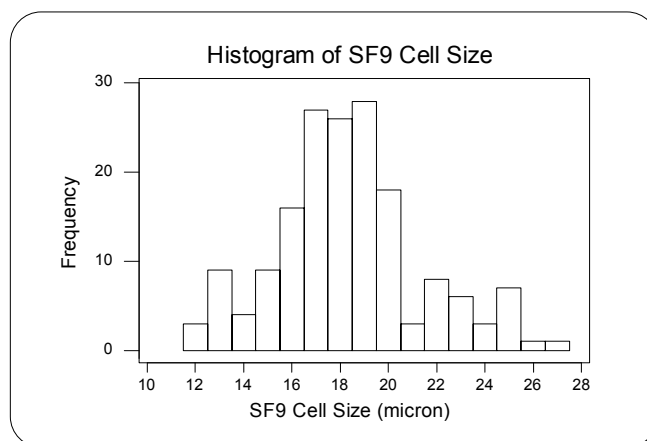


Figure 3: Cell size histogram of SF9 cells measured by Cellometer Auto T4.

Cell size analysis is used to monitor SF9 cultures before and after infection. Multiple cell size data can be displayed on the same histogram. SF9 cell images and cell size comparison histogram before and after infection are shown in Figure 4.

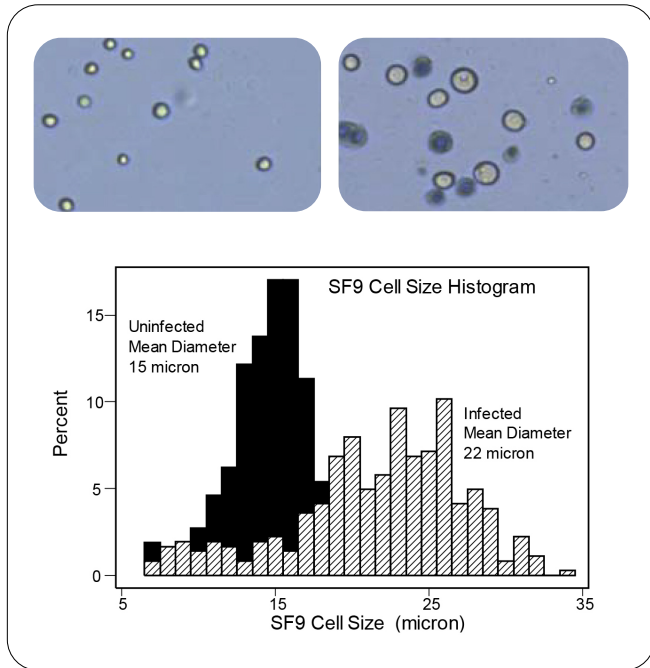


Figure 4: Cell size histogram of SF9 cells measured by Cellometer Auto T4 before and after infection.

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