HELA CELL COUNTING BY OPTICAL DENSITY MEASUREMENTS WITH PHOTOPETTE®

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- *Photopette® was used within the cell culture hood to count cell numbers.*
- *By using a custom or the HeLa cell calibration of this application note, cells can be counted within seconds.*

OBJECTIVE

The objective of this application note is to demonstrate how Photopette® can be used for measurement of cell number by optical density. Advantageously, Photopette® enables cell number measurements directly in the cell culture hood. This application note demonstrated cell number measurements of HeLa cell lines, how to prepare a calibration curve and to determine the biomass in the cell suspension. Furthermore, it acts as a handy guide to get you started with Photopette®, and outlines application-specific parameters for reference.

INTRODUCTION

Many cell culture experiments require accurate counting of cell number, either for plating of cells at a given density, for determining the rate of cell proliferation or to determine the best time for cell harvesting. Counts of live cells are routinely performed using either a hemocytometer or Coulter counter. The use of hemocytometers is very tedious and time consuming and Coulter counters require large capital investment. Other methods have been reported for quantification of cell number by measurement of cellular components [1][2][3]; however, those methods are equally complex to perform.

An alternative method is the indirect measurement of cell numbers by measuring the optical density of cell suspensions. Cell suspensions are turbid. Cells absorb and scatter light. The higher the cell concentration, the higher the turbidity. Spectrophotometers can measure intensity of light very accurately to determine turbidity. The cell culture is placed in a transparent cuvette and the light absorption is measured relative to cell culture medium alone. Optical density is directly proportional to the biomass in the cell suspension in a given range that is specific to the cell type.

In this application note, we use Photopette® Bio or Cell at 570 nm to quantify the cell number in HeLa cell cultures. By using sterile CuveTips™, measurements can be performed within seconds directly in the cell culture flask within the hood.

MATERIALS AND APPARATUS

Instruments:

- Photopette® Bio/Cell with 570 nm wavelength
- Sterile CuveTip™

Cell line:

- HeLa Cell Line human, Sigma-Aldrich

METHOD

Highlighted in this section are the steps to perform cell culture and steps before counting cell with Photopette®. The following protocol is for culturing HeLa cells that are ≥ 80% confluent in a 75 ml cell culture flask.

Aspirate off the cell medium, Dulbecco's Modified Eagle Medium (DMEM). Wash the cells with 5 mL PBS. Aspirate off the PBS. Add 3 mL of Trypsin/EDTA. This is just enough to cover the surface of the flask. Return cells to incubator for ~5 minutes. Add 9 mL of cell medium to the flask. The FBS quenches the trypsinization. Pipette medium up and down, and squirt it around the surface of the flask to remove adherent cells from the surface. Transfer ~12 mL of the cell suspension into a 15 mL Falcon tube. Spin down the cells at 1000 rpm for 5 minutes. Aspirate off the supernatant and re-suspend the HeLa cell in 1 ml DMEM medium. Count the Hela cell with a INCYTO™ C-Chip™ disposable hemocytometer. Then dilute the HeLa cell with DMEM by serial dilution within a range of several thousand to several million cells per mL.

SAMPLE EXPERIMENT

Turn-on the Photopette® device and connect to the Photopette® iOS/Android app. Select 'Cell counting' as the measurement type. Select dataset and set additional settings (if needed) before selecting *'Start Measurement'*. Please refer to the video-tutorials on the Tip Biosystems

webpage (www.tipbiosystems.com) to get familiar with the measurement process.

The blank sample was prepared by double distilled water. A CuveTip™ was placed firmly onto the device probe and dipped into the blank sample to perform the auto-zero measurement. Please ensure that there is no air-bubble trapped in the CuveTip™ cavity. Presence of air-bubbles may disrupt the optical-path and can create errors. Alternative, the blank measurement can be performed in the cell culture media (without cells present).

Subsequently, the above prepared HeLa cell suspensions with concentrations ranging from 5,860 cells/ml to 5,860,000 cells/ml were measured. The absorbance for HeLa cell was measured using a Photopette® device and five repeats were taken for each sample. The results of the measurements at 570 nm are tabulated in Table 1.

Cell Density *(cells/ml)*	Average Absorbance *(AU)*	*Standard Deviation (AU)*
5,860,000	2.968	0.005
2,930,000	2.179	0.010
1,465,000	1.646	0.006
586,000	1.424	0.007
293,000	1.190	0.004
146,500	1.060	0.002
58,600	1.045	0.008
29,300	1.027	0.001
14,650	1.022	0.007
5,860	0.964	0.006

Table 1: HeLa cell absorbance measured by Photopette®.

The absorbance values for other wavelengths (600 nm and 630 nm) and different suspension solution were also recorded and are presented in Figure 1.

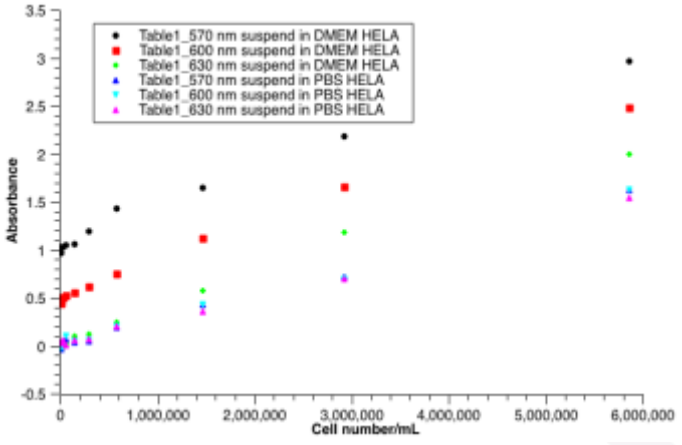

Fig 1: Optical density measurement of HeLa cell by Photopette®.

The wavelength of 570 nm gave the largest slope and was selected for the calibration. The different absorbance values at a cell number of zero are contributed to different absorbance of the media alone. By using the cell culture media for the auto-zero measurement an absorbance of zero for a sample without cells can be obtained for all different wavelengths. The data of Table 1 was used for generation of a standard curve and to determine experimental parameters such as limit of detection, linear range and dynamic range.

STANDARD CURVE

The data obtained with the Photopette can be easily loaded into Microsoft Excel® or other software by using the data export function in the Photopette® app. A standard curve was plotted in Figure 2 for cell concentration of up to 6,000,000 cells/mL. A linear regression was performed on the data using Microsoft Excel® software and the equation of the standard curve along with its R-square value was obtained.

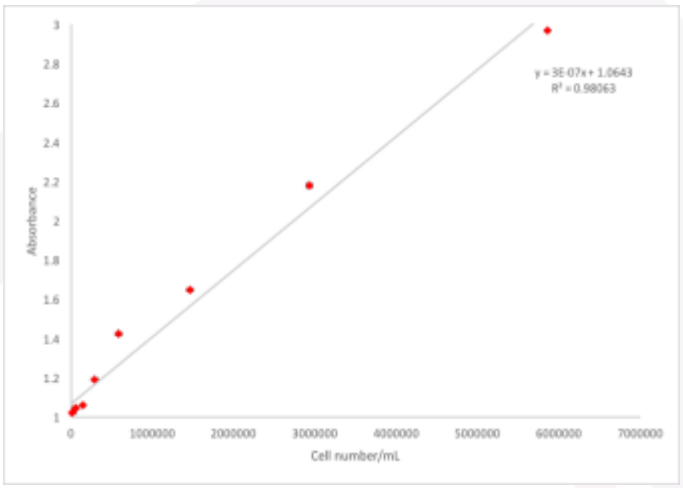

Fig 2: Standard Curve for HeLa cell counting by Photopette® for a wavelength of 570 nm.

Photopette® users may download a pre-configured worksheet for cell counting experiment from our online resource section. The worksheet is compatible with Microsoft Excel® and similar worksheet software, and will aid users in performing the calculations and generating the standard curve.

DETERMING THE CELL DENSITY OF AN UNKNOWN SAMPLE

Following a similar approach, absorbance of unknown sample can be conveniently measured using Photopette®

devices. The cell number can be calculated by substituting the absorbance value in the equation of the standard curve.

For the example above, the cell number for an unknown sample is calculated as:

$$(cells/ml) = (AU - 1.0643)/10^{-7}$$

For example, a measurement of an absorbance of 1.220 AU at 570 nm will be equivalent to 519,000 cells per milliliter.

DISCUSSIONS

LIMITATIONS

The absorbance value is affected by the suspension medium. Therefore, different cell culture or suspension solution like DMEM and PBS buffer will give different calibration curves. However, this error can be corrected by performing the auto-zero measurement in the respective medium/solution. The sloop of the calibration is not effected by the media type. It is noticed that the slope of the calibration curve in Figure 2 is larger for small cell densities compared to large high cell densities. For more accurate calibration a nonlinear curve fit can be performed.

For highest accuracy, a custom calibration of the respective cell line must be performed. The calibration curve will depend on the cell size and morphology which may be affected by the type of media and the cell culture parameters. For an estimate of the cell number for HeLa cells, the calibration in this application note can be used.

SUMMARY

The Photopette® Bio or Cell enables fast and convenient cell counting by performing ansorbance measurements at 570 nm. The Photopette app has an inbuild application for this measurement. Advantageously, measurements are directly performed within the hood and without sample transfer by using sterile CuveTips™. A dynamic range from 6 thousand to 6 million cells per milliliter was obtained.

REFERENCES

[1] **Gillies, R.J., N. Didier and M. Denton.** 1986. Determination of cell number in monolayer culture. Anal. Biochem. *159*:109-113.

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[3] **Lewinsohn, D.M., B.J. Nickoloff and E.C. Butcher.** 1988. A fluorometric approach to the quantitation of cell number with application to a cell adhesion assay. J. Immunol. Methods *110*:93-100. 7.