SHIMADZU
Excellence in Science

Application News

Dynamic Particle Image Analysis System iSpect™ DIA-10

Enumeration and Morphological Evaluation of Spheroids (Cell Clusters) by iSpect DIA-10

Hiroki Maeda and Mika Okada

User Benefits

- It is possible to quickly detect almost all particles in the sample solution (for a 100 µL sample solution, it takes a few minutes).
- With the autofocus function that does not require a standard sample, focusing can be completed in about 15 seconds.
- By filtering based on shape parameters, it is possible to count spheroids only, even in the presence of single cells mixed in the sample.

■ Introduction

Spheroids (cell clusters) are cellular aggregates produced by a three-dimensional culture, unlike a conventional two-dimensional culture. Cell clusters such as spheroids exhibit physiological functions closer to those of living organisms than adherent cells produced by two-dimensional cultures, making them useful for cell therapy and drug sensitivity testing. Understanding the concentration and size of spheroids is important for investigation of their culture conditions, quality control, and research applications.

The Shimadzu iSpect™ DIA-10 dynamic particle image analysis system (Fig. 1), which is based on the dynamic image analysis method, is an instrument that acquires images of particles in liquid samples and measures the particle size distribution, concentration, and shape. With an optical system that misses very few particles and has an image acquisition efficiency of 90% or higher, it is possible to analyze tens of thousands of particles and extract and count spheroids by setting filtering conditions.

This article introduces an example in which suspensions of HEK293 single cells and spheroids were evaluated using the iSpect DIA-10.

Fig. 1 iSpect™ DIA-10 Dynamic Particle Image Analysis System

■ Samples and Methods

A liquid containing single cells and spheroids of HEK293 cells suspended in a DMEM medium was used as the sample. The measurement conditions are shown in Table 1. To evaluate cell clusters larger than 100 µm, measurements were taken using a flow cell with a channel thickness of 300 µm (custom order required).

Table 1 Measurement Conditions

System	iSpect DIA-10
Flow Cell	300 µm cell*
Frame Rate [fps]	6
Efficiency [%]	90.5
Pump Volume [µL]	50
Pump Rate [mL/min]	0.2
Luminance	240
Threshold of Binary Image	220

* Custom order required. This is used when samples contain particles larger than 100 µm. However, the detection performance of particles less than 20 µm decreases.

■ Results

Fig. 2 shows the state of the flow cell during the measurement. Particles that are considered to be spheroids, single cells, and contaminants can be observed.

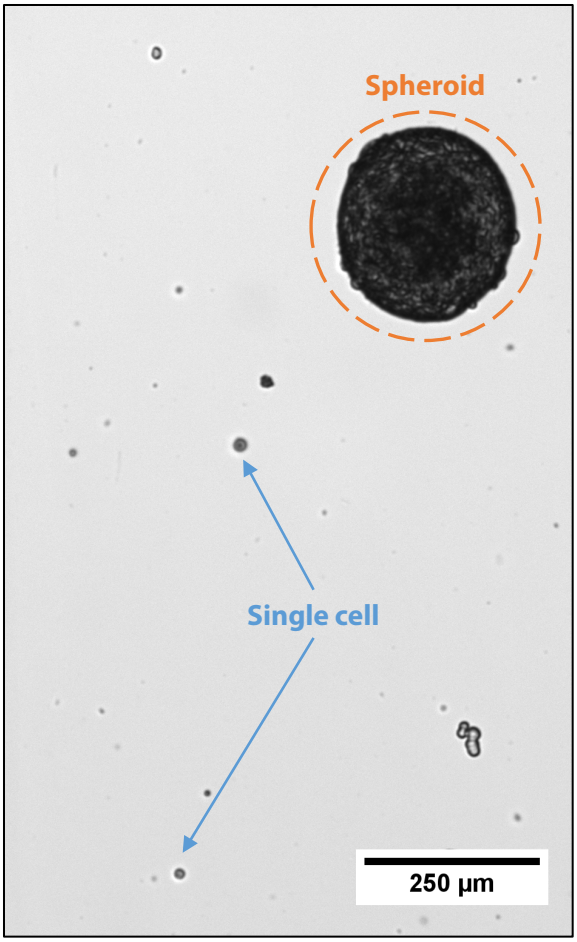

Fig. 2 Representative Image of the Flow Cell during the Measurement
This shows one of the 90 images captured during the measurement.

Figs. 3 and 4 show the scatter plot and the particle size distribution, respectively. From the scatter plot, two groups of particles can be identified: Group 1 (shown in blue) with a distribution of roughly 5-50 µm and Group 2 (shown in orange) with a distribution of roughly 80-400 µm. Furthermore, the particle size distribution shows many particles in the range of approximately 5-50 µm.

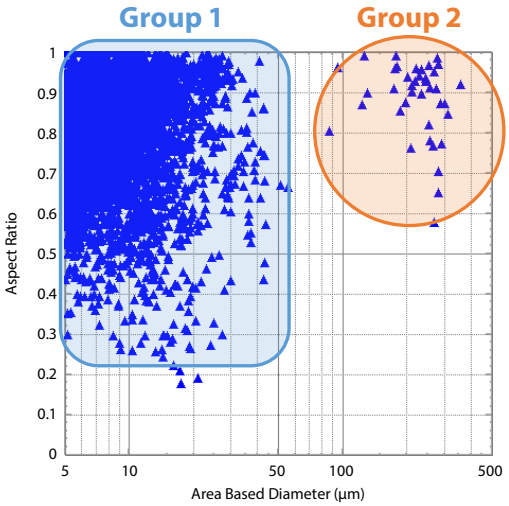

Fig. 3 Scatter plot
X-axis: Area Based Diameter Y-axis: Aspect Ratio*
*[Aspect Ratio] = [Maximum Length] / [Pattern Width]

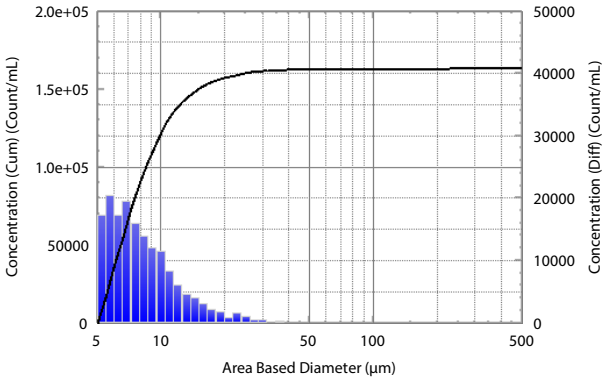

Fig. 4 Particle size Distribution

Fig. 5 shows a part of the particle images, arranged in order of their area-based diameters in decreasing size from the top left, and displaying particles with diameters ranging from approximately 40-220 µm. Based on the particle images, particles larger than or equal to 86.907 µm are spheroids. In comparison, particles smaller than or equal to 55.503 µm are considered single cells, clusters of single cells, or debris. Therefore, particles larger than 60 µm are considered to be almost entirely spheroids, and the particle concentration for each particle size range was obtained and listed in Table 2. Using particle images and shape parameters for filtering, only particles considered spheroids were extracted, and their number concentration could be determined.

Table 2 Concentration

	Concentration [Count/mL]
Group 1 (< 60 µm, single cells or debris)	163055
Group 2 (≥ 60 µm, spheroids)	862

■ Conclusion

Using the iSpect DIA-10, a suspension of HEK293 cells containing both single cells and spheroids was measured. By analyzing the scatter plot, particle size distribution, and particle images, we could identify the region where spheroids were present and filter out other particles, enabling us to obtain the concentration of spheroids. Even for mixed samples, it is possible to count only the particles of interest by filtering using particle size and shape parameters. Additionally, the iSpect DIA-10 can detect tens of thousands of particles in just a few minutes and requires only a small amount of sample, making it efficient for counting spheroids.

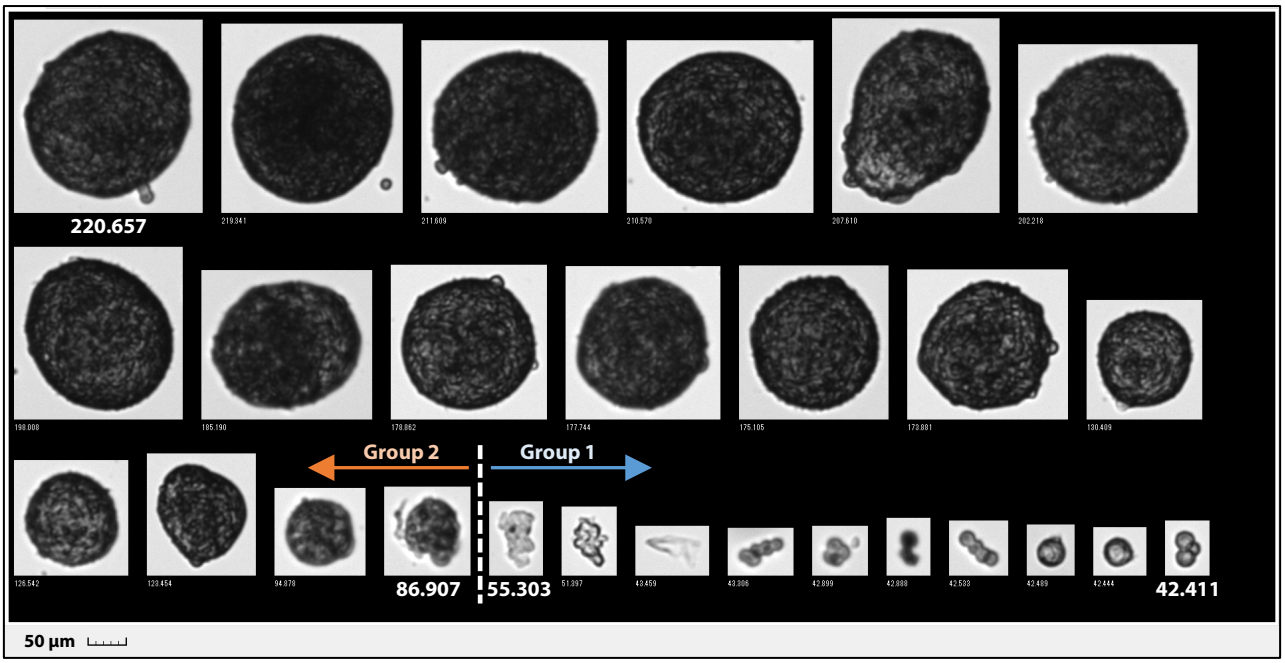

Fig. 5 Particle Images
The particles are arranged in order of their area based diameters in decreasing size from the top left,
and display particles with diameters ranging from approximately 40-220 µm.
The values below each particle image represent the area based diameter (µm).

01-00430-EN First Edition: May 2023

› Please fill out the survey

Related Products Some products may be updated to newer models.

› iSpect DIA-10
Dynamic Particle Image Analysis System

Related Solutions

› Biopharmaceutical

› Cell culture / Production control

› Price Inquiry

› Product Inquiry

› Technical Service / Support Inquiry

› Other Inquiry