

APPLICATION FOCUS: CELLOMETER VISION

Automated Counting and Sizing Freshly Isolated Adipocytes with Minimal Sample Preparation

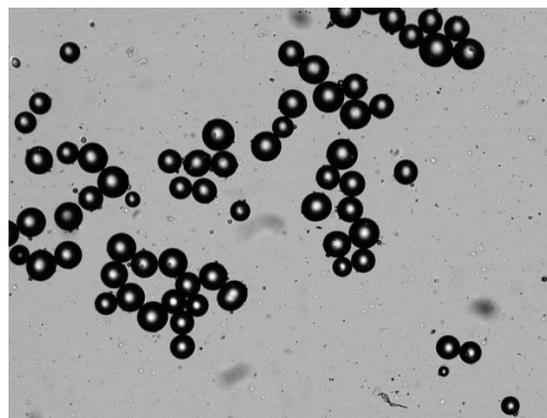


INTRODUCTION

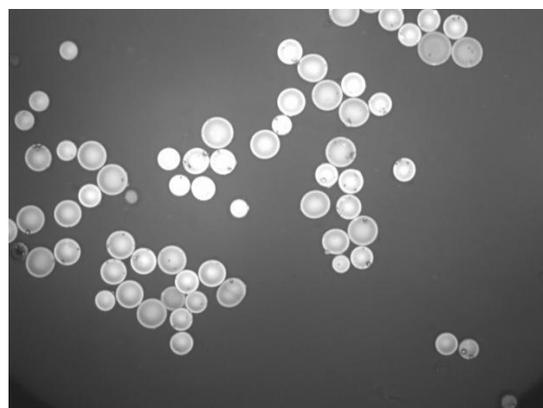
Cellometer Vision incorporates image based cell counting and fluorescence detection in a compact and easy-to-use instrument. With fluorescence detection capabilities, Cellometer Vision is an ideal solution for counting complex samples with minimal sample preparation, such as counting and sizing adipocytes.

The size and number of adipocytes are directly associated with adipose tissue metabolism. However, currently available automatic cell counting methods often require complex sample preparation and use of hazardous reagents. With a traditional manual hemacytometer method, there is a technical challenge in distinguishing intact adipocytes from lipid droplets generated from ruptured adipocytes in the collagenase digestion process. Cellometer Vision incorporates both total cell counting and fluorescence detection in one easy-to-use instrument. By instantly staining enzymatic treated fat tissue samples with acridine orange (AO), a DNA fluorescence dye, Cellometer Vision can detect and analyze fluorescent positive adipocytes. In less than a minute, the system can generate accurate cell counting results and also report cell sizes. Various primary adipocytes, such as human, mice, rat, etc. with diameters of 25um to 250um can be easily loaded into a disposable counting chamber designed especially for adipocytes to eliminate liquid handling reliability issues.

Using proprietary algorithms, Cellometer Vision's robust operating software accurately analyzes cell images, and generates counting data in less than 60 seconds. Cell images and all analysis data, including cell size distribution histograms, can be saved for documentation. Data can also be easily exported to Microsoft Excel spreadsheets for further analysis.



Brightfield Image of adipocyte sample. Lipid droplets are indistinguishable from intact adipocytes.



Fluorescent image showing AO stained adipocytes. Lipid droplets are not visible.

Counting Results	
Sample: Adipocyte_AO	
Dilution: 1.0	
Assay: Adipocytes_AO	
Description: Adipocytes stained with acridine orange	
Cell: Adipocytes_AO	
Description:	
	Bright Field Fluorescence
Count	63 56
Mean Size	89.6
Concentration	9.98×10^{-4} 8.89×10^{-4}
$\frac{\text{F1 Count}}{\text{BR Total Count}} = 88.9\%$	
Export	Show Size Distribution
Print	Sample Adjustment
	Set Data File
	Save to Data File
	View Data File
	Done

Counting results box displays adipocyte cell count, concentration and size data.

METHOD

Direct AO labeling of adipocytes:

1. Pipette 90µl of adipocyte cell sample into a microtube.
2. Apply 10µl of 20 µg/ml acridine orange solution to adipocyte cell sample.
3. Mix cell sample with acridine orange solution gently.
4. Load 55µl of labeled sample into the PD300 deepwell disposable counting chamber.

Running Assay:

1. Load 20µl of labeled sample into the disposable counting chamber.
2. Insert chamber into Cellometer Vision.
3. Select assay from drop-down menu.
4. Enter sample ID for this sample.
5. Preview cell images and click 'Count' to begin analyzing sample.
6. Review images and counting results.
7. Save or export images and/or report data.

RESULTS

Both adipocytes and lipid droplets are visible in the brightfield mode (Figure 1) while AO stained adipocytes are clearly visible in the acquired fluorescence image (Figure 2), distinguishing them from lipid droplets. The software indicates counted cells with green circles (enlarged to show detail). Results display (Figure 3) indicates counted cells, as well as mean size, and automatically calculates concentration.

Cell size data (Figure 4) and distribution histograms (Figure 5) can be easily generated. All experimental data can be instantly transferred to an Excel spreadsheet or saved in a data table.

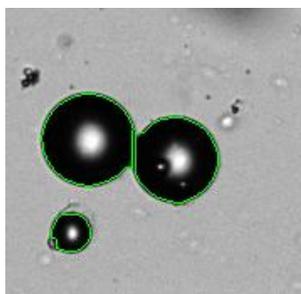


Figure 1. Brightfield image showing adipocytes and lipid droplets.

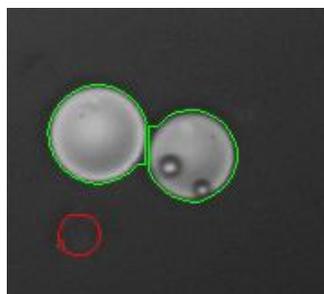


Figure 2. Fluorescent image showing counted AO stained adipocytes. Lipid droplets are not counted and indicated by red circle.

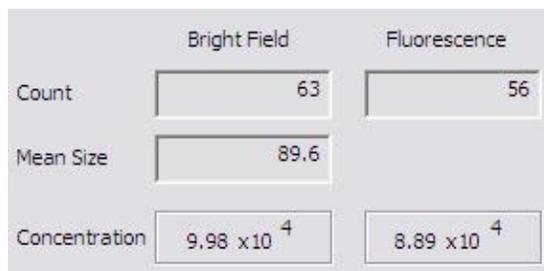


Figure 3. Total count (Brightfield) and adipocyte only count (Fluorescence) as well as adipocyte mean size are indicated on-screen immediately after image analysis.

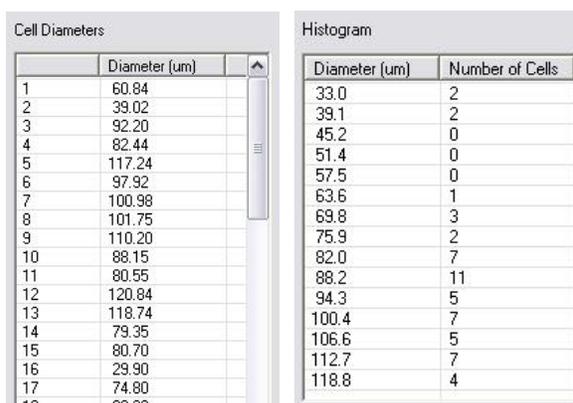


Figure 4. Cell diameter data can be easily displayed or exported to Excel for further analysis.

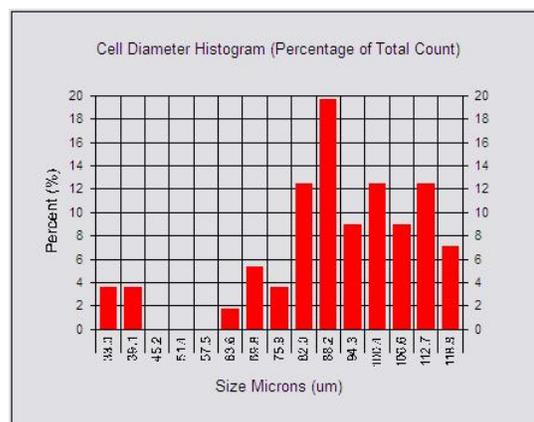


Figure 5. Adipocyte size distribution histogram displayed on-screen.

CELLOMETER Vision Trio SPECIFICATIONS:

Imaging Modes: Brightfield & 2 Fluorescence Channels

Filter Set 101: Excitation/Emission Peak: 475nm/535nm

Filter Set 202: Excitation/Emission Peak: 525nm /595nm

Dimensions: 6"x 8.5" x 14" (15cm x 22cm x 36cm)

Weight: 25lbs (11kg)

PC Specs: WinXP/1.8+GHz/1GB RAM/laptop included