ZETAVIEW® PARTICLEMETRIX

Operator (Report): ZetaView
Video Operator: ZetaView

1 — Sample Parameters

Sample Name: ExoSpin Isolated Exosomes
Comment: SN215, CA0032, cell0127, offset=-3, T=no, Sens85, Sh70, MinB20, MinS5, MaxS200
Electrolyte: PBS, Concentration: 0.000 N
Temperature: 27.09 °C sensed
pH 7.0 entered
Conductivity: 12092.05 µS/cm sensed

2 — Result (sizes in nm)

	Number	Concentration	Volume
Median (X50)	79.0	79.0	109.4
Span	32.6	32.6	41.4

Concentration:	5.1E+7 Particles / mL
Dilution Factor:	700
Original Concentration:	3.5E+10 Particles / cm³

3 — Measurement Parameters

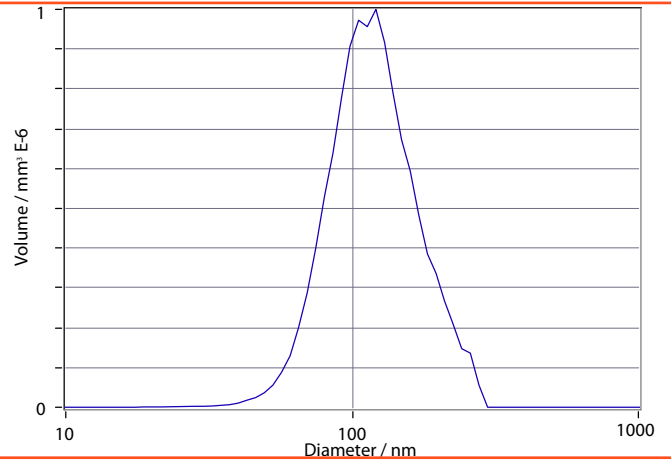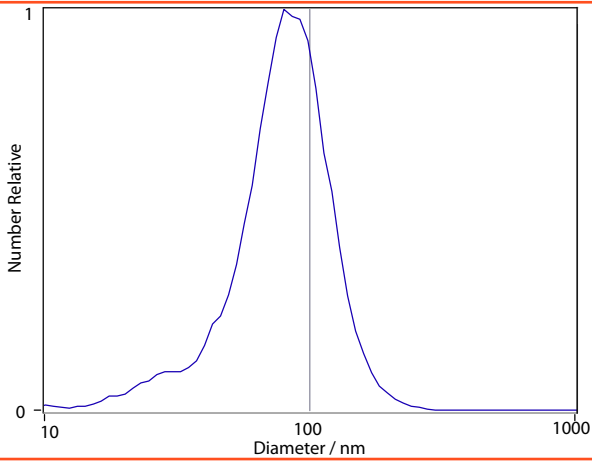Cell S/N: CA0032-0141b

4 — Quality

Average Counted Particles per Frame: 170
Number of Traced Particles: 1024

5 — Measurement Mode: Size Distribution 2 Cycles

11 Positions, 2 Removed for Analysis

6 — Analysis Parameters

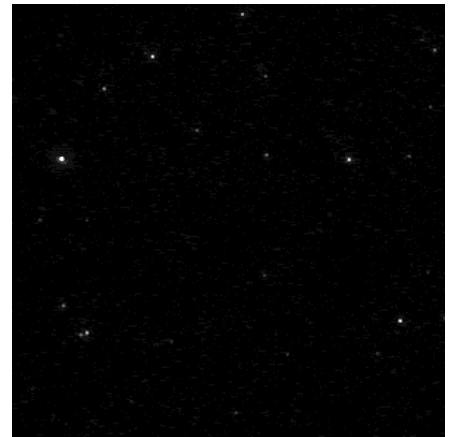Max Size: 1000, Min Size: 10, Min Brightness: 20

7

8

10

9 — Peak Analysis (Number Absolute)

Diameter / nm	Number Absolute	FWHM / nm	Percentage
85.1	78.0	65.8	100.0

11 — X Values

	Number	Concentration	Volume
X10	42.5	42.5	73.3
X50	79.0	79.0	109.4
X90	118.6	118.6	173.7
Span	1.0	1.0	0.9
Mean	83.4	83.4	121.1
StdDev	32.6	32.6	41.4

12 — Comment

13

AB

(Signature)

A guide to understanding the ZetaView analysis report

Cell Guidance Systems

Version 1.0 (May 2016)

1. Sample parameters
   a. **Sample name**: entered by the operator.
   b. **Comment**: based on instrument settings selected by the operator.
   c. **Electrolyte**: entered by the operator.
   d. **Temperature**: sensed by the instrument.
   e. **pH**: entered by the operator.
   f. **Conductivity**: Sensed by the instrument.

2. Results
   a. **Median**: see explanation on quantiles in section 11 below
   b. **Number**: The median based on the measured size of the particles in the "number weighted distribution". In the example, this means that the middle number of all readings is 79 nm. The number indicates the median value.
   c. **Concentration**: Median based on the measured concentration of the particles in the "concentration weighted distribution". In the example, this means that the middle number of all readings is also 79 nm (because concentration derives from the number)
   d. **Volume:** Median based on the measured volume of the particles in the "volume weighted distribution". In this example, this means that the middle number of all readings (volume of the particles) is $109.4mm^3$E-6
   e. **Span:** is a statistical value that provides a measure of the distribution width. It is similar to FWHM (full width half maximum) but is calculated differently. The equation underlying this value is (X90-X10)/X50. Since the shape of the distribution is equal for "number weighted and concentration weighted distribution", the values are equal too (32.6nm).

3. Measurement parameters
   Represents the serial number of the Cell Assembly – cell combination. It provides information which cell calibration file was used during the measurements.

4. Quality
   a. **Average counted particles per frame**: provides the average number of particles that are counted in one single video frame. Since there are several frames in one video (Low, mid, high, highest; corresponds to 15, 30, 60, 90 frames) the instrument calculates an average number of the particles which are counted in every single frame of the video.
   b. Not all particles are traced. The total number of particles which had movement traced is indicated.

Number of traced particles: describes the number of particles which could be detected and traced in the entire video and which are used in the analysis. Hence, the system detects a specific number of traces (e.g. 170), which correspond directly to the particles that fulfill the requirements for going into the analysis. Factors that determine the number of traced particles are:
(i). the particles must be detectable according to the adjusted "trace length" (can be adjusted in the administrator).
(ii). Particles must not disappear (out of focus or out of the window) when the adjusted trace length is not fulfilled. For example, if the trace length is adjusted to 15 (default) and some particles disappear before they were traced 15 steps, these particles will not be included in the analysis.

5. Measurement mode
   Different measurement modes are available. In our lab, we routinely use 11 positions with 2 cycles. Statistical analysis of the measurements at each position is used to identify outlier measurements. These outliers are removed from analysis.

6. Analysis Parameters
   These are set to only measure particles that fall within desired size limits defined by the operator. Please note: Max Size and Min Size do not represent the size in nanometers. These values define the number pixels for particles visible in the window.

7. The relative number of particles of each diameter is plotted

8. The volume of the particles of each diameter is plotted. Each particle of a given diameter has a specific volume. Here, the diameter for each particle is assigned to its detected volume.

9. Peak Analysis

   This is also referred to as the mode and represents the peak of frequency distribution. It indicates the size/size-range of the particles that are most commonly measured in the distribution. Here, the size of the particles that are most frequently (78 particles) measured is 85.1 nm. Furthermore, you find the FWHM (full width half maximum) which is a measure of the width of the size distribution. The percentage (100) tells you that there is only one peak in your distribution. If there is a second peak, this will also be measure resulting in two percentage values which sum to 100%.

10. A single frame captured from the video is shown.

11. Section 2 provides data on the measurements at X50

    The Quantiles X10, X50 and X90 are shown.

    X10: describes the diameter of the measured particles where 10% of the distribution has a smaller particle size and 90% has a larger particle size. In this case 10% of the particles have a size below and 90% above 55 nm in number weighted and concentration weighted

distribution. In the volume weighted distribution 10% of the particles have lower and 90% a larger size than 99.3 nm.
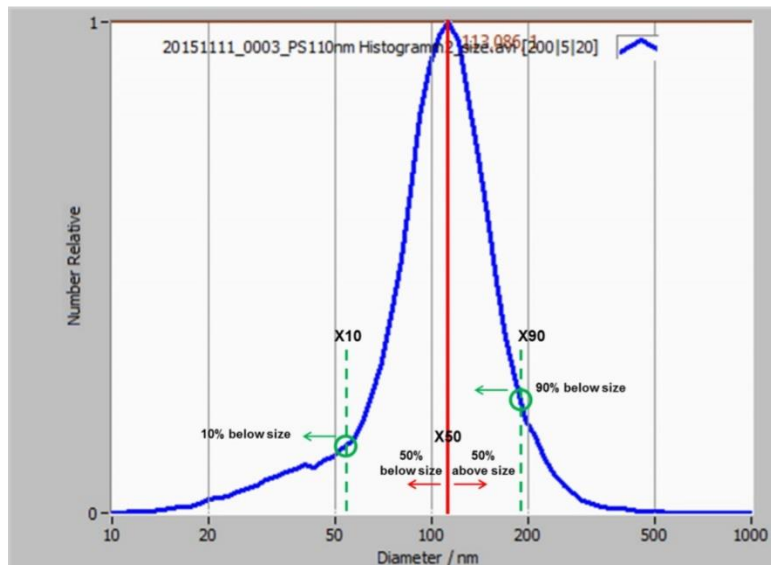

FIG The Quantiles X10, X50 and X90 are shown in the histogram. Please note: In an ideal symmetrical size distribution X10 and X90 is located on equal level.

X50: describes the diameter of the particles where 50% of the distribution has a smaller and 50% has a larger particle size. Here, 50% of the distribution has a size below and above 104.1 nm in number weighted and concentration weighted distribution. In the volume weighted distribution 50% of the particles have lower and larger size than 153.2 nm. The X50 is also called statistical median and represents the middle number in a sequence of numbers. To find the median, each number is organized by size and the number in the middle is the median. An important attribute of the median is its robustness against outliers.

X90: describes the diameter of the particles where 90% of the distribution has smaller and 10% has larger particle size. In this example, 90% of the distribution has a size below and 10% above 159 nm in number-weighted and concentration-weighted distribution. In the volume weighted-distribution 90% of the particles have lower and 10% larger size than 291.9 nm.

12. Comment field for operator. Left blank if no comment.

13. Signature of Operator.

14. Location of video used in analysis.

15. Hardware used for analysis.

16. Experimental Log.