




# Particle Metrix ZetaView Operating Training

楊忠霖


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## Particle Metrix GmbH



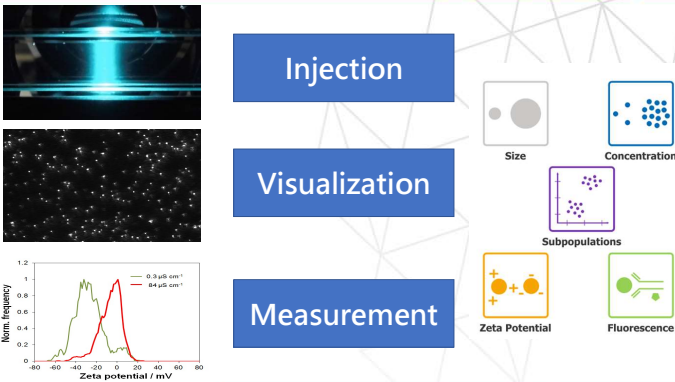
PMX-120      PMX-220      PMX-420



Particle Metrix GmbH 2004



## Nanoparticle Tracking Analysis

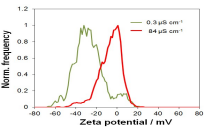


**Injection**

**Visualization**

**Measurement**

- Size
- Concentration
- Subpopulations
- Zeta Potential
- Fluorescence




Norm. frequency

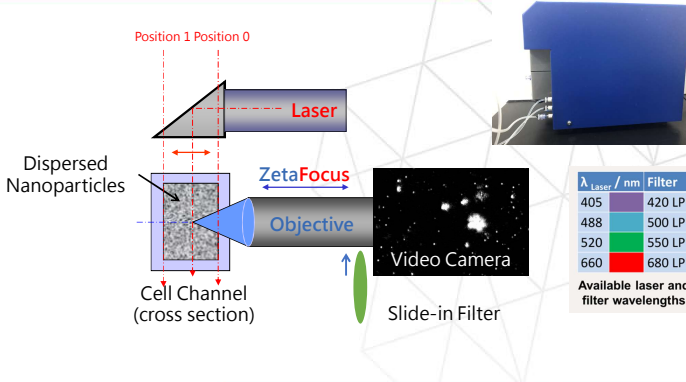
Zeta potential / mV

0.3  $\mu\text{g}/\text{cm}^3$

0.4  $\mu\text{g}/\text{cm}^3$



## Nanoparticle Tracking Analysis - Setup



Position 1 Position 0

Laser

ZetaFocus

Objective

Dispersed Nanoparticles


Cell Channel (cross section)

Video Camera

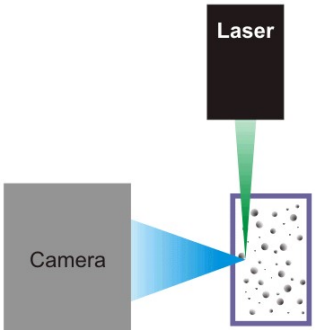
Slide-in Filter

$\lambda_{\text{Laser}} / \text{nm}$	Filter
405	420 LP
488	500 LP
520	550 LP
660	680 LP

Available laser and filter wavelengths



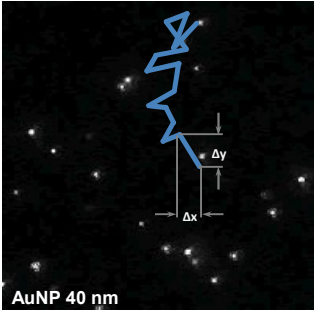
### Unique Scanning technology



- Automated measurement of 11 positions per sample
- Representative sample result with high statistical power

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### Size Measurement- Brownian Motion



AuNP 40 nm

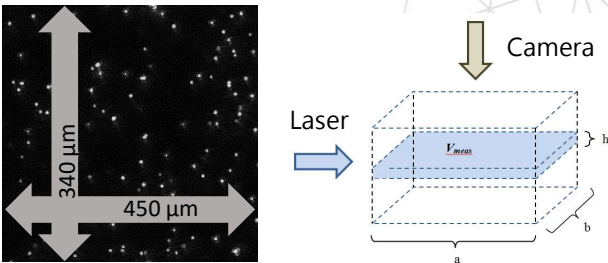
#### Stokes-Einstein

$$D = \frac{k_B T}{6\pi\eta r}$$

$D$  = Diffusion coefficient [m<sup>2</sup>/s]  
 $k_B$  = Boltzmann constant  
 $T$  = Temperature  
 $\eta$  = Viscosity  
 $r$  = Particle radius

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### Concentration Measurement



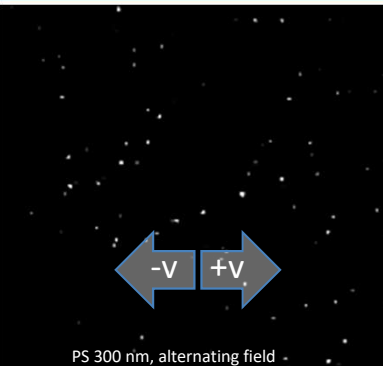
340 μm  
450 μm

Camera  
Laser

Measurement volume: ~ 3nl/position

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### Zeta Potential Measurement



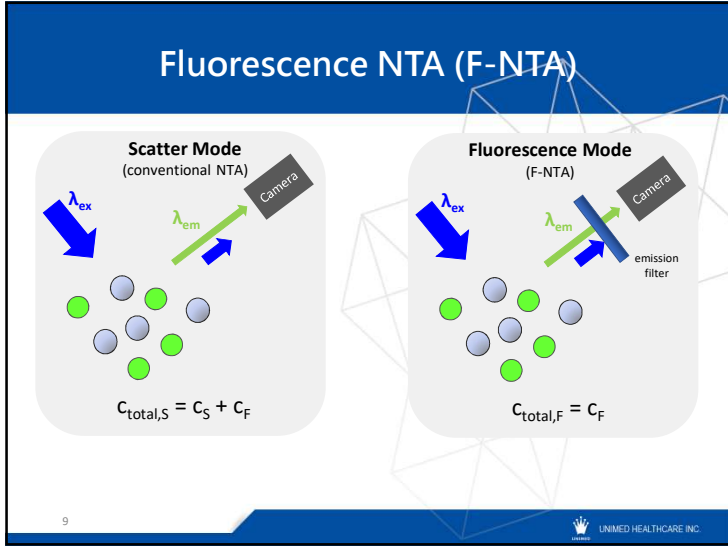
PS 300 nm, alternating field

#### Helmholtz-Smoluchowski

$$\zeta = \frac{4\pi\eta}{\epsilon} f(\kappa a) \cdot \mu_e$$

$\epsilon$  = Dielectric constant  
 $\eta$  = Viscosity of medium  
 $f(\kappa a)$  = Debye function  
 $\zeta$  = Zeta potential  
 $\mu_e$  = Electrophoretic mobility

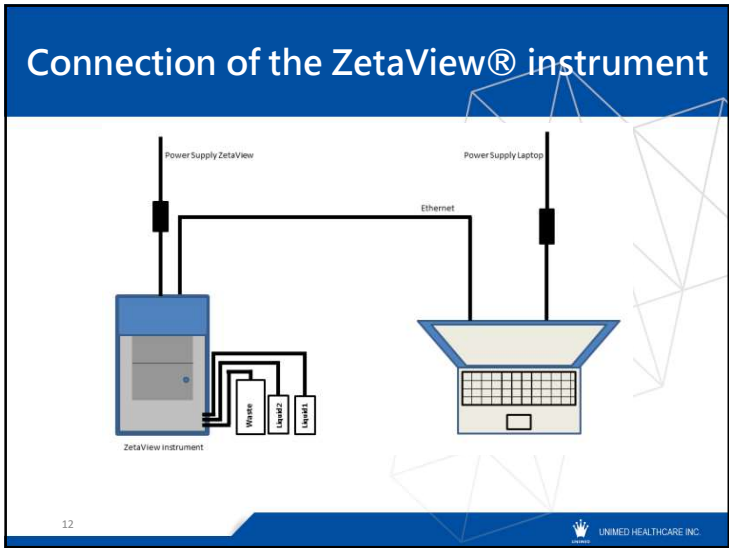
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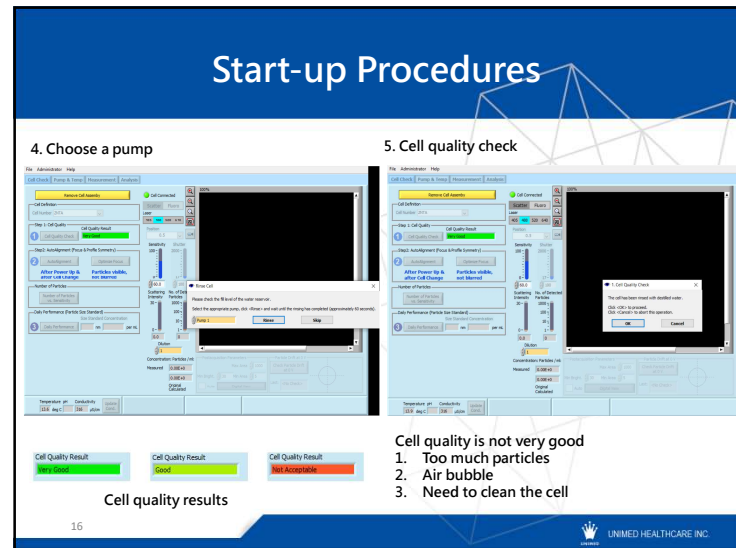
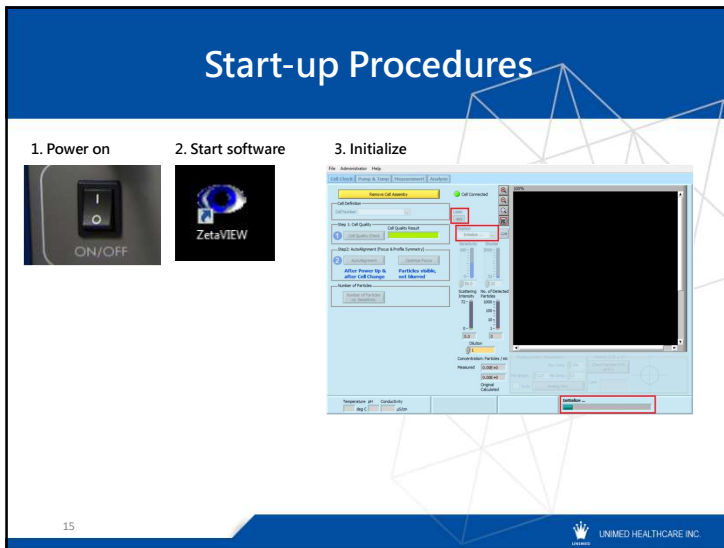
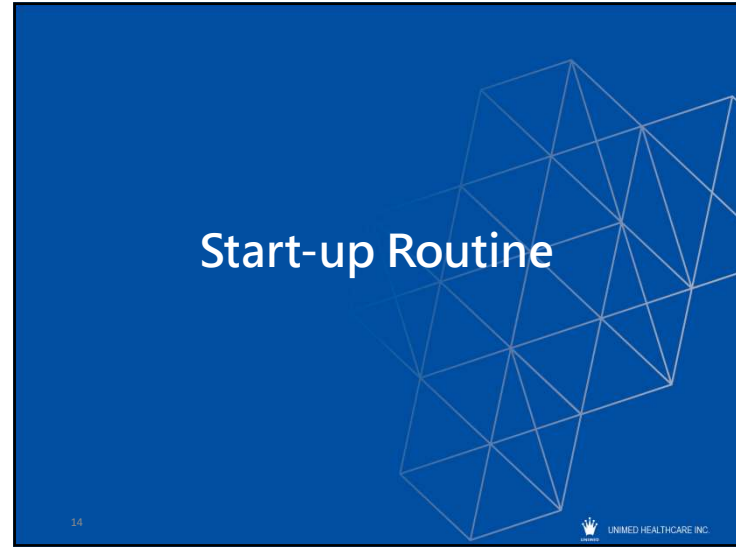
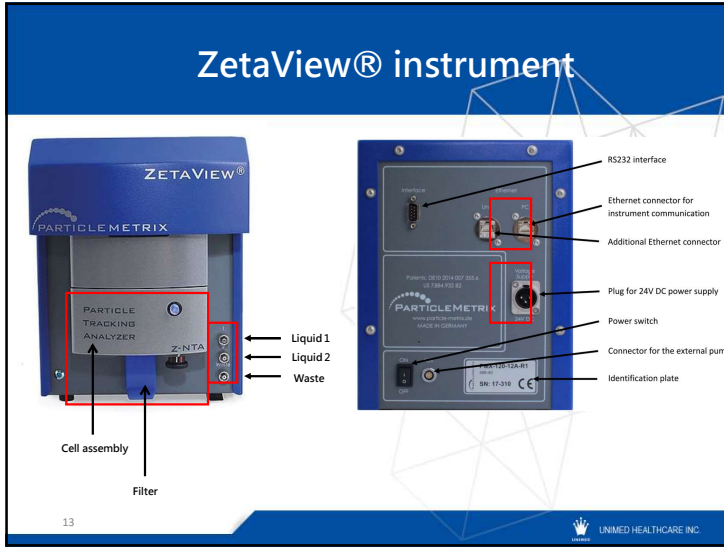


- ### Typical applications of the ZetaView
- ✓ Bionanoparticles
  - ✓ F-labeled bioNP
  - ✓ Liposomes + Micelles
  - ✓ Drug delivery
  - ✓ Proteine agglomeration
  - ✓ Virus like particles (VLP)
  - ✓ Virus samples / Viral titer
  - ✓ Extracellular vesicles (EV)
  - ✓ Emulsions
  - ✓ Polymers
  - ✓ Nanometals
  - ✓ Nanobubbles
  - ✓ Irregular particles
  - ✓ Quantum dots
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# ZetaView System

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## Standard Beads and Injection

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## Autoalignment

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## Measurements

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## Interface

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# Measurement

# Measurement - Experiment Parameters

# Data of 11 positions

Site	Position	Mean Int.	Ao. No. of Particles	Conc. (g./cm <sup>3</sup> )	Disp. Conc. (g./cm <sup>3</sup> )*3	No. of Frames	RSD (%)	Peak σ (nm)	Span	DRIFT (μm/s)	Removal
X	0.10	241	47.9	1.46E-7	4.38E-7	10	110.4	15.9	1.2	18.1	
X	0.15	243	53.0	1.51E-7	4.53E-7	11	100.5	22.9	0.8	15.4	GRUBBS_SIZE
X	0.20	245	43.0	1.43E-7	4.29E-7	20	100.0	14.5	0.8	18.1	
X	0.30	243	46.0	1.46E-7	4.38E-7	15	100.5	18.1	1.4	20.8	
X	0.40	244	76.0	1.46E-7	4.38E-7	10	100.0	18.1	0.8	18.1	GRUBBS_SIZE
X	0.50	245	86.0	1.46E-7	4.38E-7	20	119.3	14.4	0.8	16.5	
X	0.60	244	106.0	1.46E-7	4.38E-7	20	128.7	14.6	0.8	14.5	
X	0.70	244	116.0	1.46E-7	4.38E-7	20	122.7	12.7	0.7	11.7	GRUBBS_SIZE
X	0.80	244	136.0	1.46E-7	4.38E-7	17	116.0	14.6	0.8	7.1	
X	0.85	244	157.0	1.46E-7	4.38E-7	16	128.3	17.6	1.0	5.4	
X	0.90	244	159.0	1.46E-7	4.38E-7	21	116.0	13.6	0.8	4.1	GRUBBS_SIZE
Mean		245	96.7	1.46E-7	4.38E-7	18.0	123.6	14.7	1.0	13.6	
Std.Dev.		0.1	43.7	6.86E-7	2.06E-6	3.7	9.1	18.4	0.2	7.6	
Max.Dev.		2.4	47.0	7.0E-7	2.10E-6	8.0	12.4	33.4	0.5	40.5	

光強度 顆粒數量/濃度 原管濃度 偵測顆粒數 主要peak 流速速度 中位數 spars<sub>average</sub>

項目	異常值項目	定義位置	說明	可能原因
1	Min_Traces	No. of Traces	偵測顆粒需要大於5顆，少於5顆即出現此訊息。	顆粒過少；樣品濃度過低。
2	Max_Drift	Drift	顆粒流動速度大於30um/sec。	速度過高；緩衝液離子濃度或粘度不同；緩衝液淨潔；管路內含過多氣泡。
3	Range_Size	Peak Diameter	偵測到小於10 nm或大於3000 nm之顆粒。	超過偵測極限，數值不再確。
4	Range_Number	Ao. No. of Particles	偵測到平均顆粒數少於10顆或大於50顆。	樣品濃度過低或過高。
5	GRUBBS_Number	Ao. No. of Particles	顆粒數量與其他位置差異過大。	樣品未平均分布。
6	GRUBBS_MI	Mean Int.	光強度與其他位置差異過大。	氣泡；顆粒過大；高可見光顆粒汙染；游離氯過剩；蛋白質汙染；緩衝液或水質樣品種(自動收品發生問題)。
7	GRUBBS_SIZE	Peak Diameter	顆粒大小與其他位置差異過大。	樣品未平均分布。

# Zeta Potential Measurement



# Exported Data

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## Exported PDF - Size

樣品及偵測資訊

圖譜 (No. / Conc.-Weight)

主要peak資訊

統計

統計資訊 (X50)

濃度

統計之粒子數量

圖譜 (Volume-Weight)

即時影像截圖 (隨機截圖)

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## Exported PDF - ZP

樣品及偵測資訊

圖譜

SL數據

統計

結果

統計之粒子數

統計

即時影像截圖 (隨機截圖)

**EVs ZP: -15~-30**  
**Lower than -30 - Bad sample quality**  
**Higher than -15 - Bad sample quality and cause aggregation**

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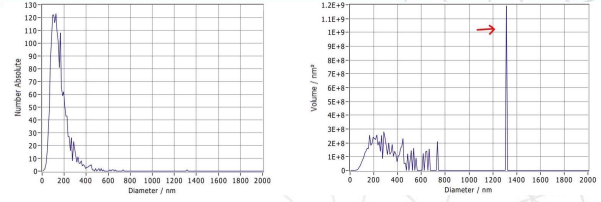
## Stokes-Einstein equation & Data graph

$$\frac{(x,y)^2}{4} = Dt = \frac{k_B T}{3\pi\eta R} t \rightarrow \text{Volume}/\text{nm}^3 = \frac{1}{6}\pi R^3$$

$Dt$  = Diffusion coefficient [m<sup>2</sup>/s]  
 $k_B$  = Boltzmann constant  
 $T$  = Temperature  
 $t$  = time (s)  
 $\eta$  = viscosity

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## Stokes-Einstein equation & Data graph



$$\text{Volume} / \text{nm}^3 = \frac{1}{6} \pi r^3 \quad (\text{The formula for the volume of a sphere})$$

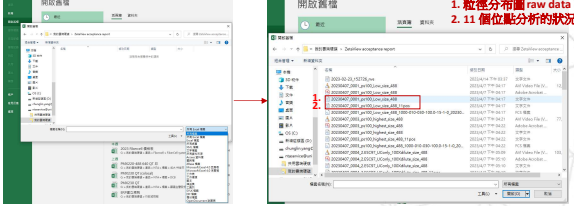
if diameter (1300 nm) =  $\frac{1}{6} \pi * 1300 * 1300 * 1300 = 1.2\text{E}+09 \text{ nm}^3 / \text{particle}$   
 if diameter(100 nm) =  $\frac{1}{6} \pi * 100 * 100 * 100 = 5.2\text{E}+06 \text{ nm}^3 / \text{particle}$

If air bubble >> check MI (Mean intensity) on 11 position information table  
 Check sample again by the video film (.avi)

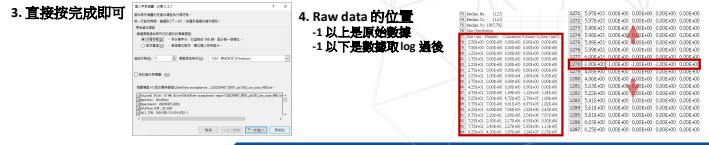
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## Exported raw data

1. 開啟 EXCEL 軟體
2. 選擇開啟檔案->選擇所有檔案->選擇要開啟的 raw data



3. 直接按完成即可
4. Raw data 的位置  
-1 以上是原始數據  
-1 以下是數據取log 過後



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# Troubleshooting

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## Excessive drift of particles

1. Drift in vertical direction
  - Heating up the measurement cell by the laser
  - The ambient temperature is too warm
  - > **Solution:** Turn on the temperature control and cool down a few degrees below the measured cell temperature.
2. Drift in horizontal direction
  - A defective check valve in the waste line or at the Cell Assembly.
  - A defective check valve does not close tightly anymore.
  - A cracked measurement cell inside the Cell Assembly.
  - Leaky tubing inside or outside the instrument.
  - Air bubbles inside the tubing
  - > **Solution :** Ensure there are no bubbles in the cell, consider changing the check valves, check the tubing, and check the measurement cell

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## Particles out of focus

1. Size of the particles. Once focused on small particles, large particles in a subsequent sample may appear to be out of focus.  
 -> Solution: Perform "Autoalignment" with PS 100nm alignment suspension or "Optimize Focus" with the particles in the sample.

Step2: AutoAlignment (Focus & Profile Symmetry) -----

AutoAlignment

After Power Up Bk after Cell Change

Optimize Focus

Particles visible, not blurred

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## Measured voltage is too low

1. Air bubbles inside the cell that prevents electrical current flow from one cell flange to the other  
 -> Solution: Flush the instrument with air and rinse it again with water and standard suspension.

Air bubble??

Cell Flange      Cell Flange

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## Preventing Maintenance and Service

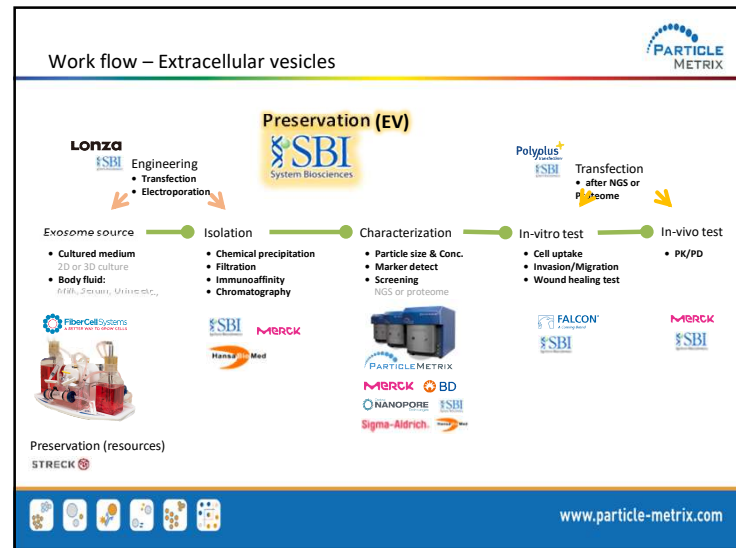
Maintenance: Every year

Pump head exchanged: Every year

Education: Depends

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 02-27202215 # 157  
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PARTICLE METRIX

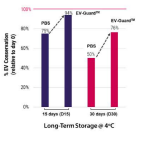
明星商品

## SBI EV-GUARD

List of Components		
Item	Catalog #	Volume
EV-Guard™ EV storage buffer – 1X	EXBA-1	40 ml
EV-Guard™ EV storage buffer – 10X concentrated	EXBA-10	4 ml

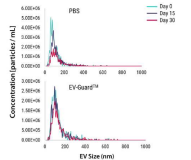


**A.**

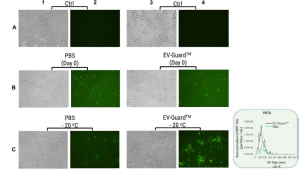


Long-Term Storage @ 4°C

**B.**



**C.**



**Figure 2:** EVs from normal human serum were isolated using SBI's ExoBead and stored at 4°C for periods of 0, 15, and 30 days. The percentage of EV conservation (A) and EV size distribution (B) were subsequently analyzed using fluorescent nanoparticle tracking analysis (NPTA).

**Figure 3:** GFP-labeled EVs were stored in either PBS (columns 1 and 2) or EV-Guard™ (columns 3 and 4) at 4°C or 22°C for 1 month before being introduced to HEK293 cells for transfection (Row C). Row A represents the negative control (no GFP EV), while Row B is the positive control (no storage). After 1 month at 22°C, the number of GFP-EVs was analyzed using fluorescent NTA (as shown in the box to the right).

[www.particle-metrix.com](http://www.particle-metrix.com)

# Discussion





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