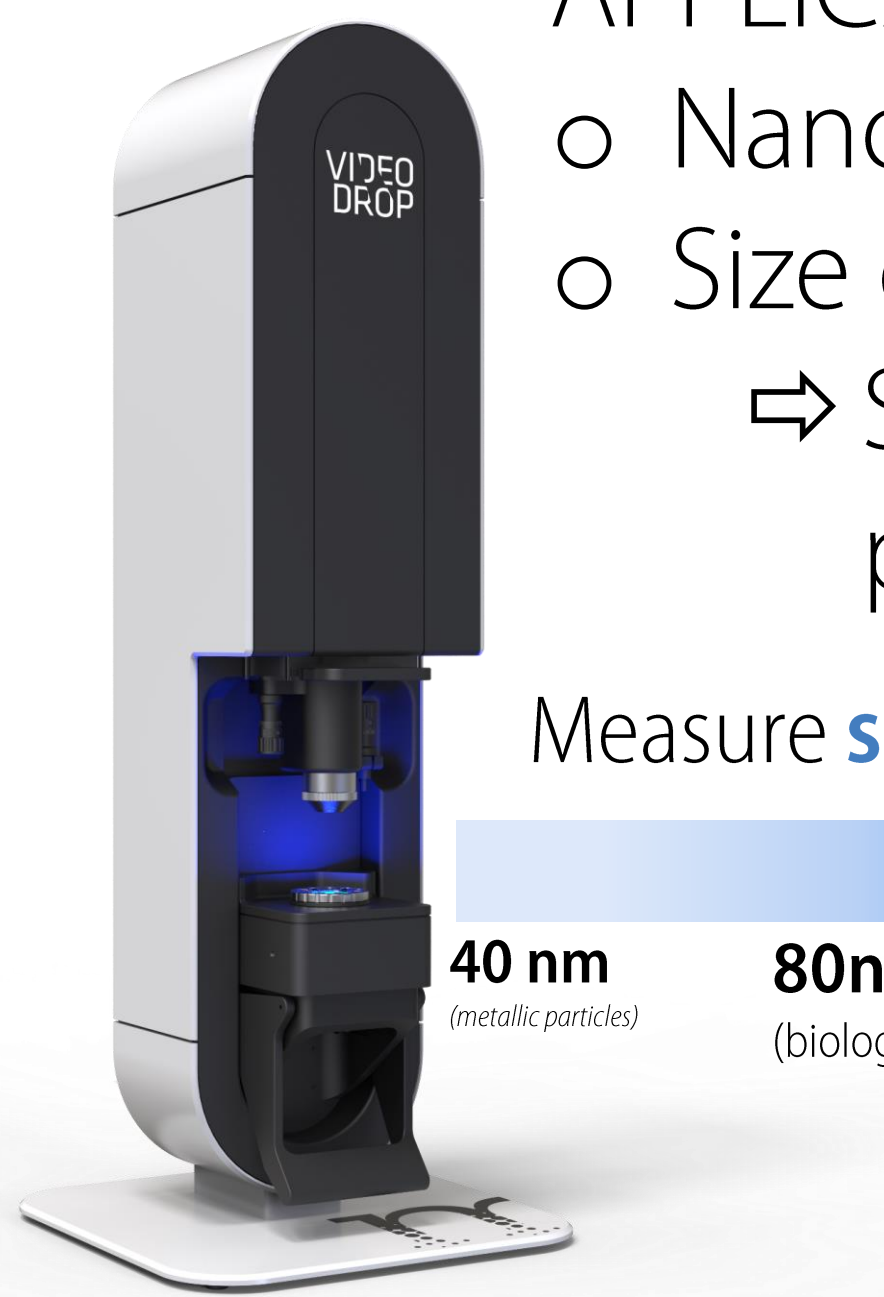


Introduction

Videodrop (ILM) is a custom microscope that uses interference phenomenon to detect the light scattered by individual nanoparticles in solution. Using this interferometric signal, nanoparticles are automatically detected and tracked to compute **concentration and hydrodynamic diameters**.

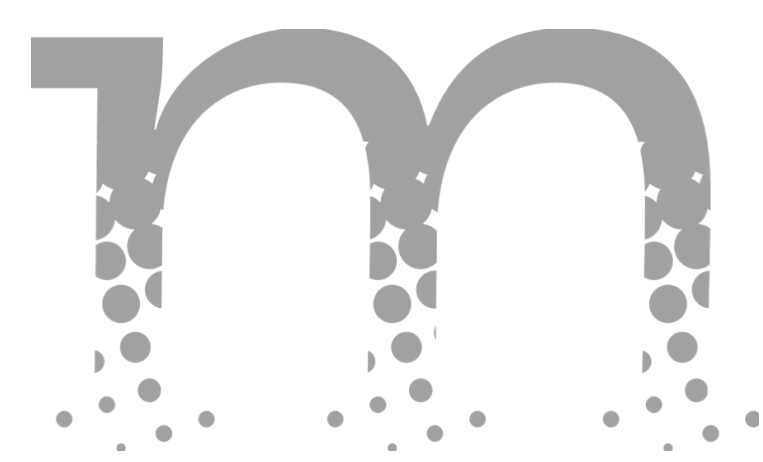
APPLICATIONS

- Nanoparticles concentration (virus, EVs)
- Size distribution
- ⇒ Suitable for in-process controls and final product characterization



Measure **size & concentration** Visualize **debris & aggregates**

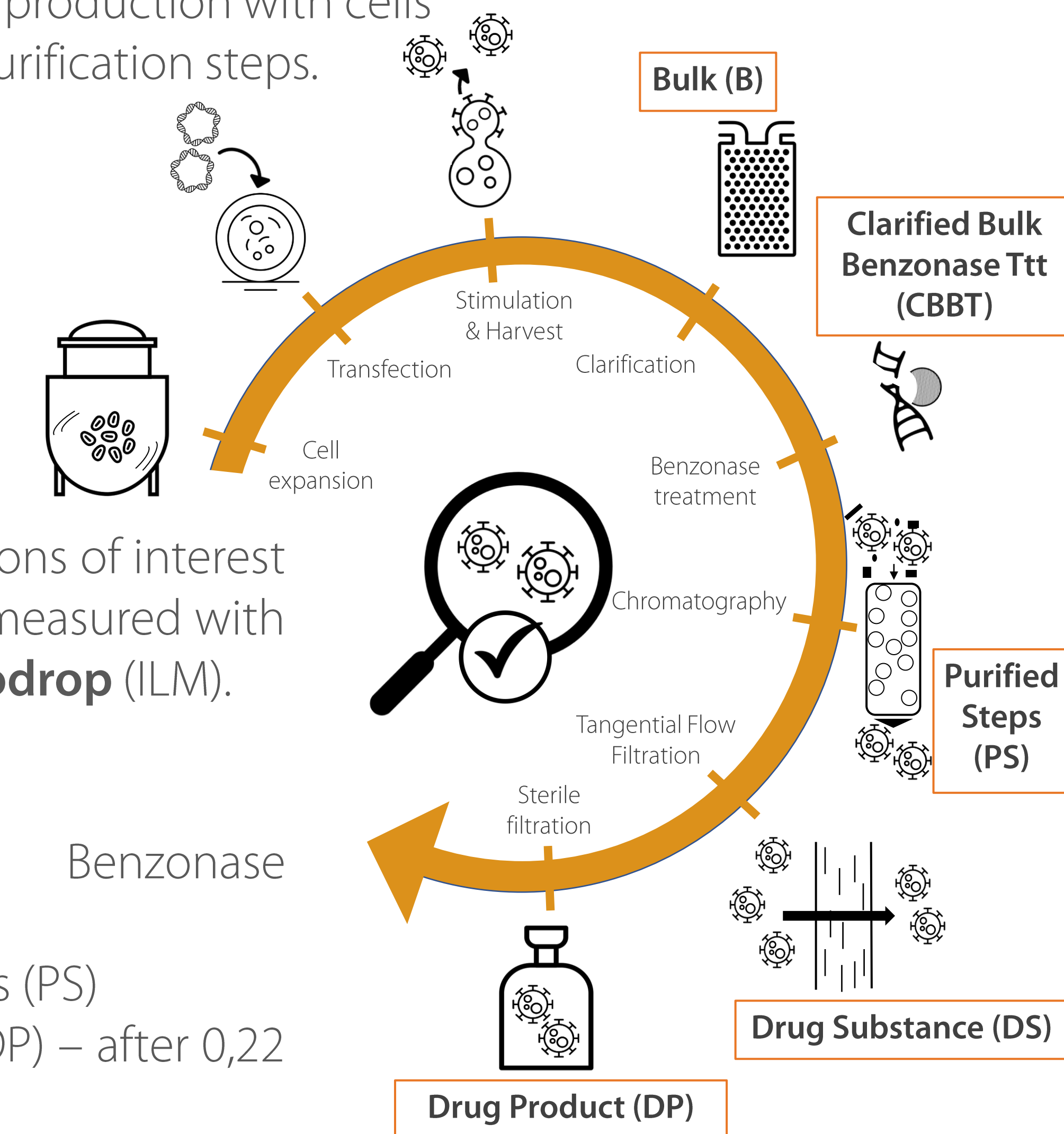
40 nm (metallic particles) 80nm (biological particles) 500 nm up to 10 µm



LENTIVIRAL PRODUCTION PROCESS

Lentiviral vectors production is divided in two main steps:

- Upstream process = virus production with cells
- Downstream process = purification steps.



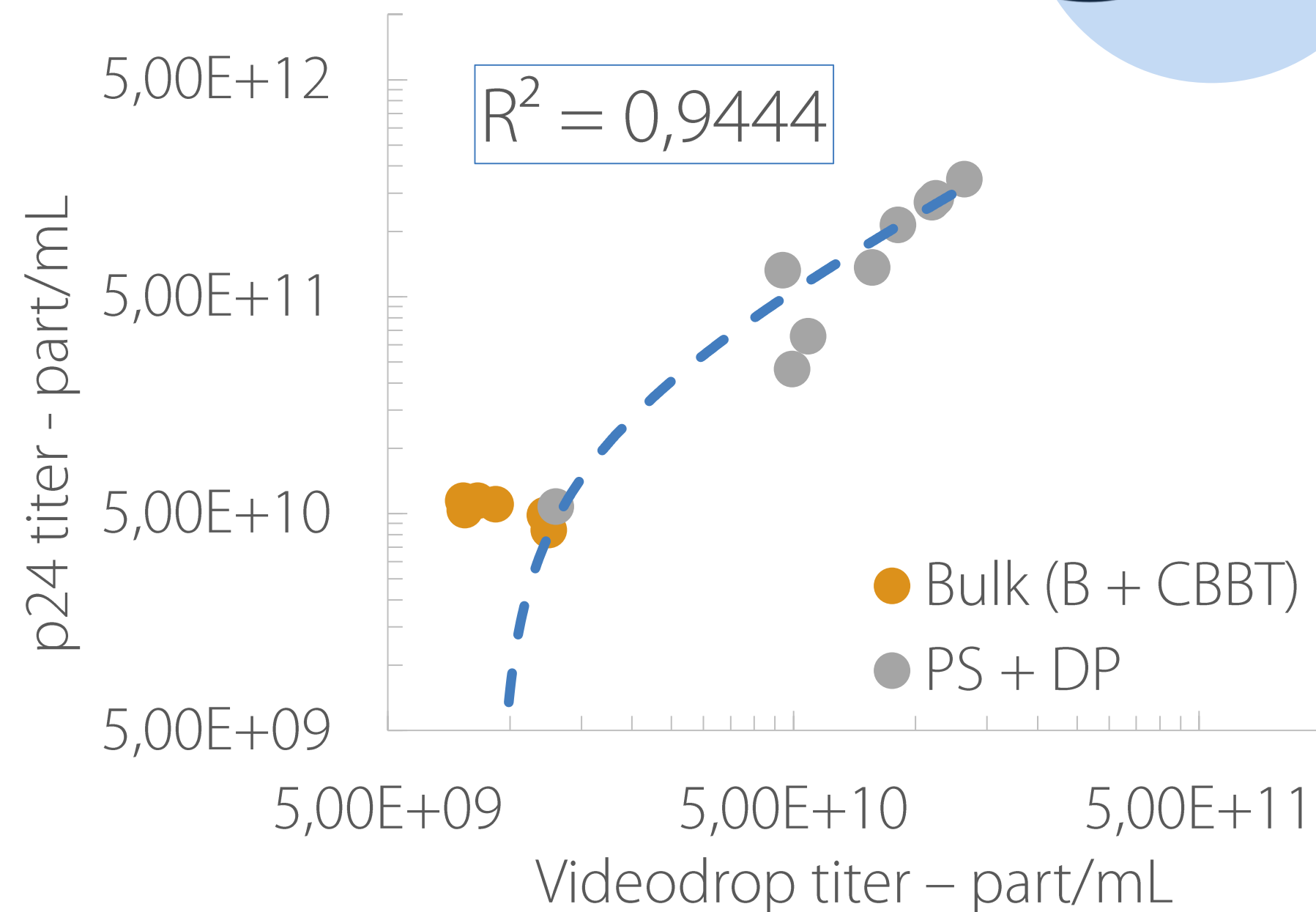
The physical titer of 5 fractions of interest in 3 different batches was measured with **p24 ELISA, NTA and Videodrop (ILM)**. The evaluated fractions are:

- Bulk (B),
- Clarified Bulk after Benzonase treatment (CBBT),
- different Purification steps (PS)
- and final drug product (DP) – after 0,22 µm filtration.

PHYSICAL TITER QUANTIFICATION METHOD COMPARISON

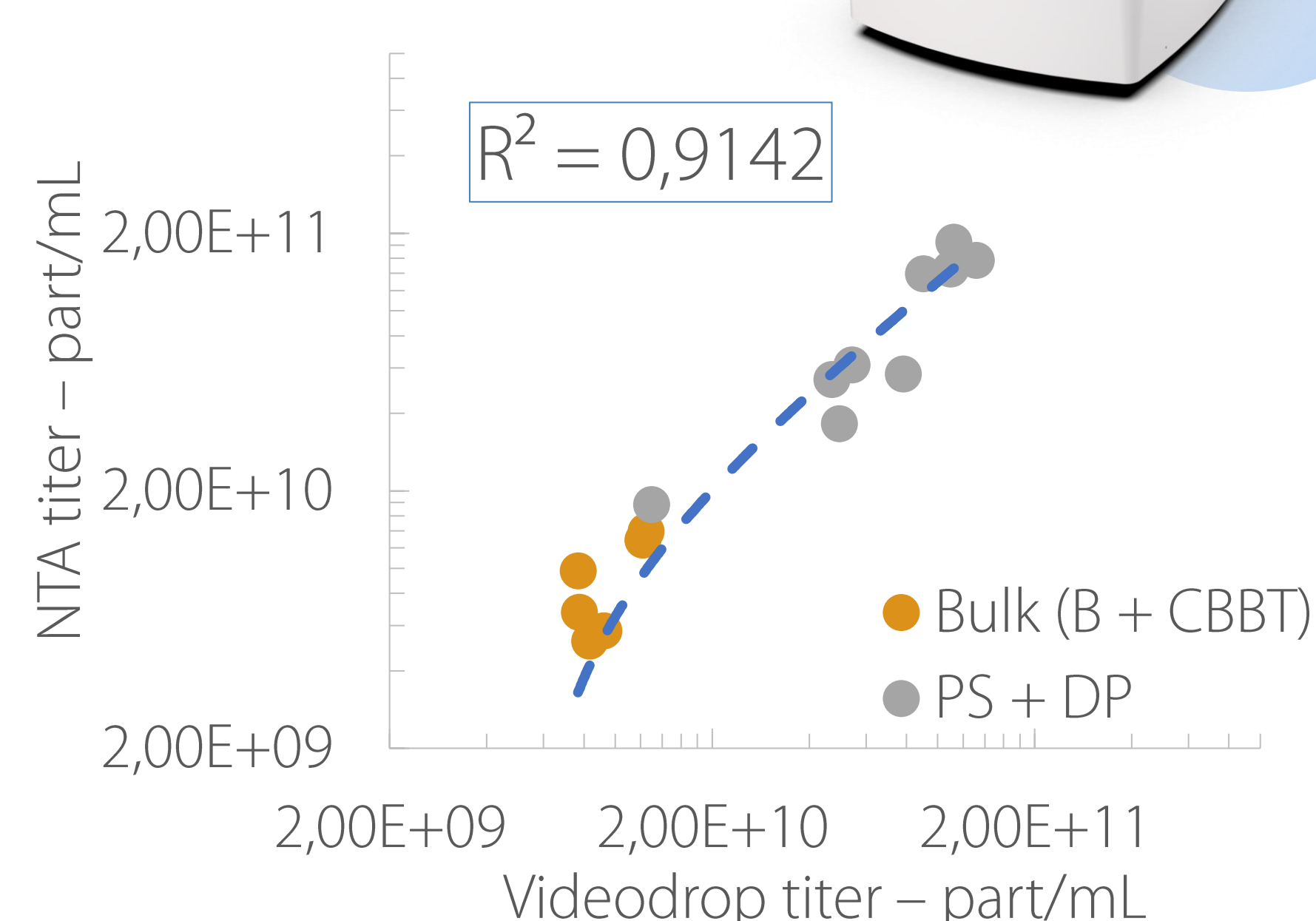
P24 ELISA

CONCENTRATION



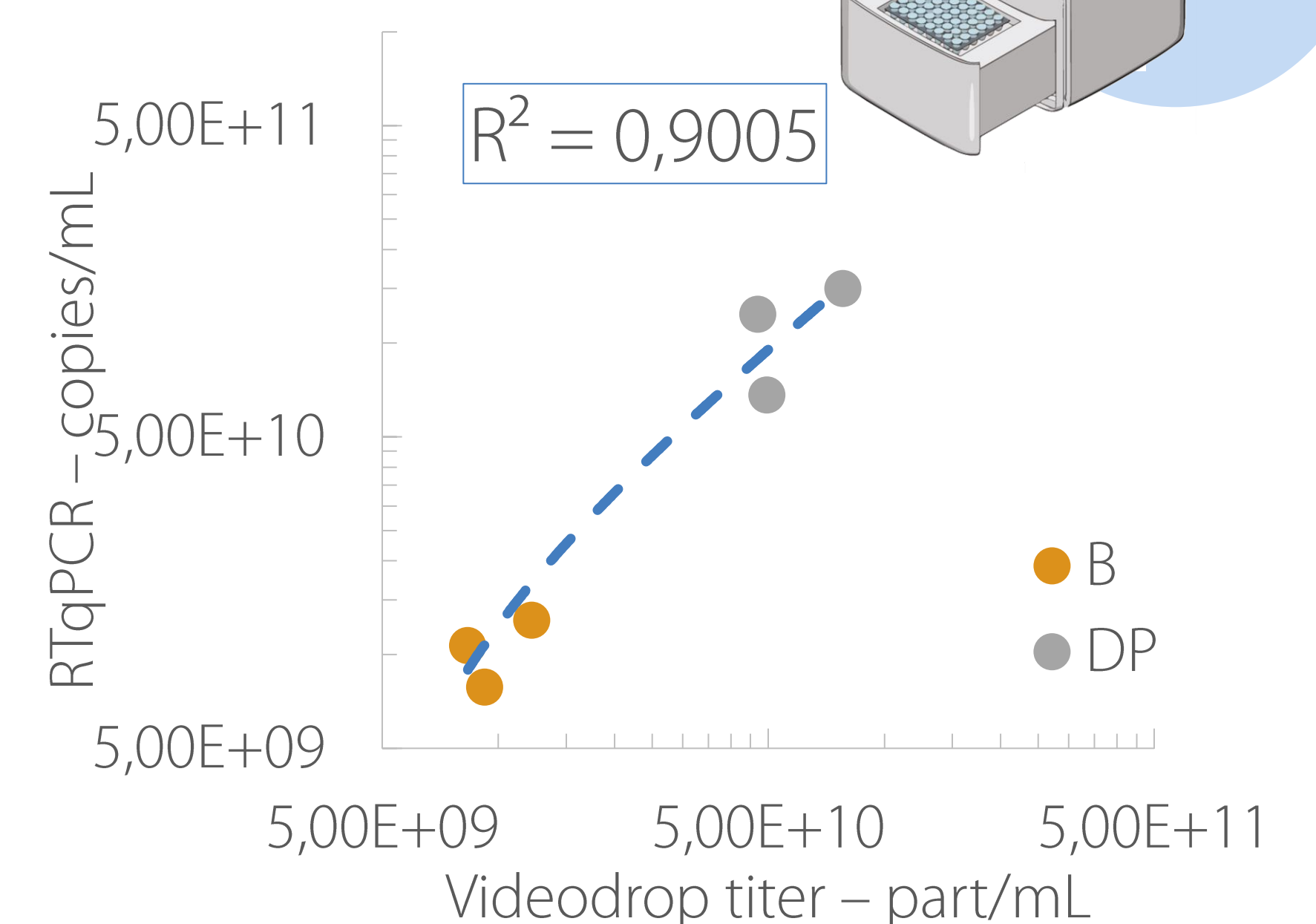
NTA

CONCENTRATION



RTqPCR

CONCENTRATION



The results of ILM (Videodrop) show a **strong correlation** with p24 ELISA ($R^2 = 0.94$), NTA ($R^2 = 0.91$) and RTqPCR titers ($R^2 = 0.90$). The results show the **suitability** of the Videodrop to be used for **physical titer determination**. The ease of use, and rapidity of the Videodrop compared to the three described techniques, make it an **ideal tool for bioproduction follow-up**.

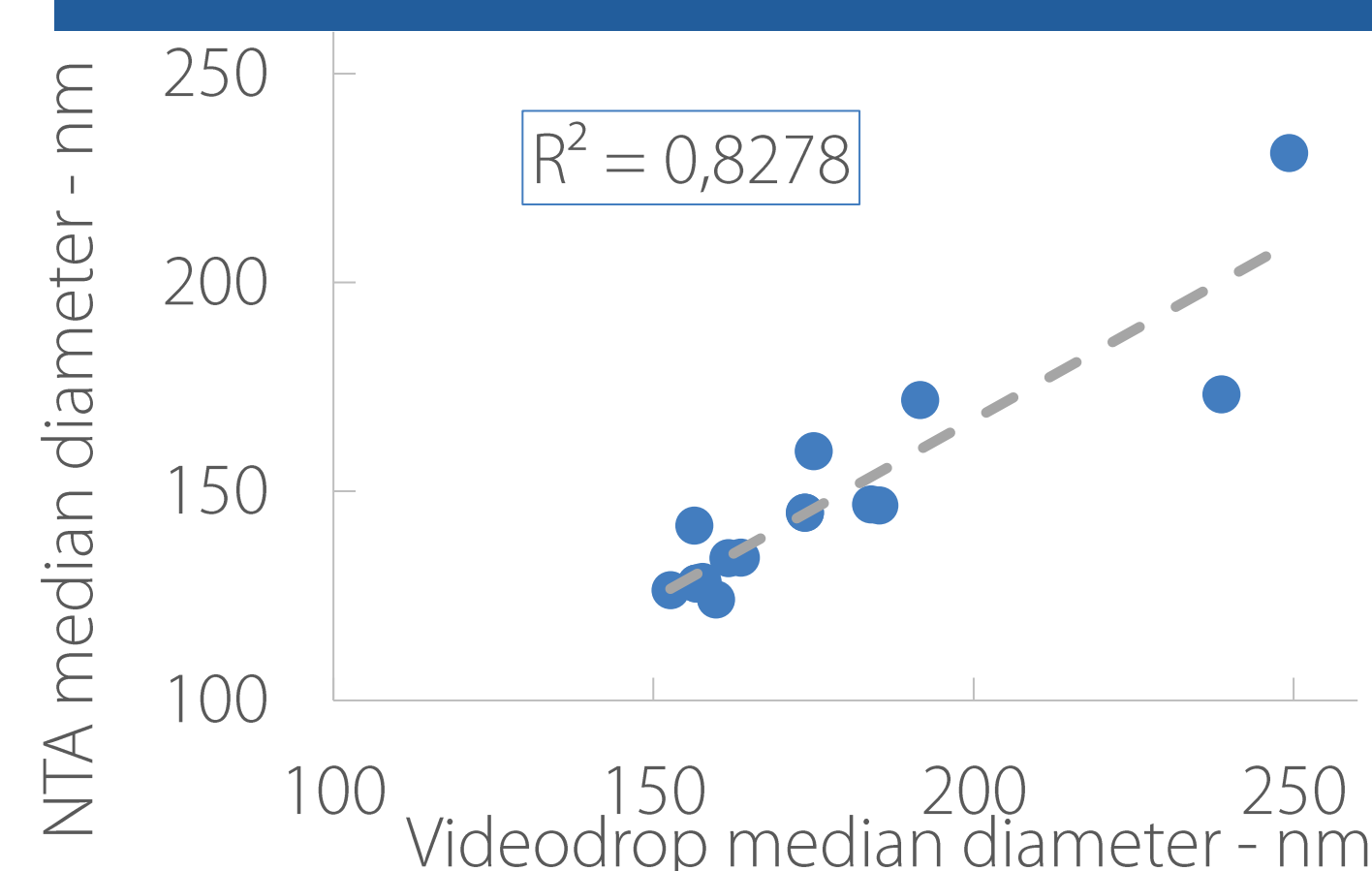
SIZE MEASUREMENT OF AGGREGATED LENTIVIRUSES

Lentiviral vector aggregation is a complex mechanism that impacts the quality of the product. In this study we propose to generate aggregated samples to challenge both abilities of **NTA and Videodrop (ILM)** to analyze complex samples.

Stress induced viral vector samples

- 2 Drug Product (DP)
- 2 temperatures : 25°C & 50°C
- 4 time points : T0, T24h, T48h, T72h

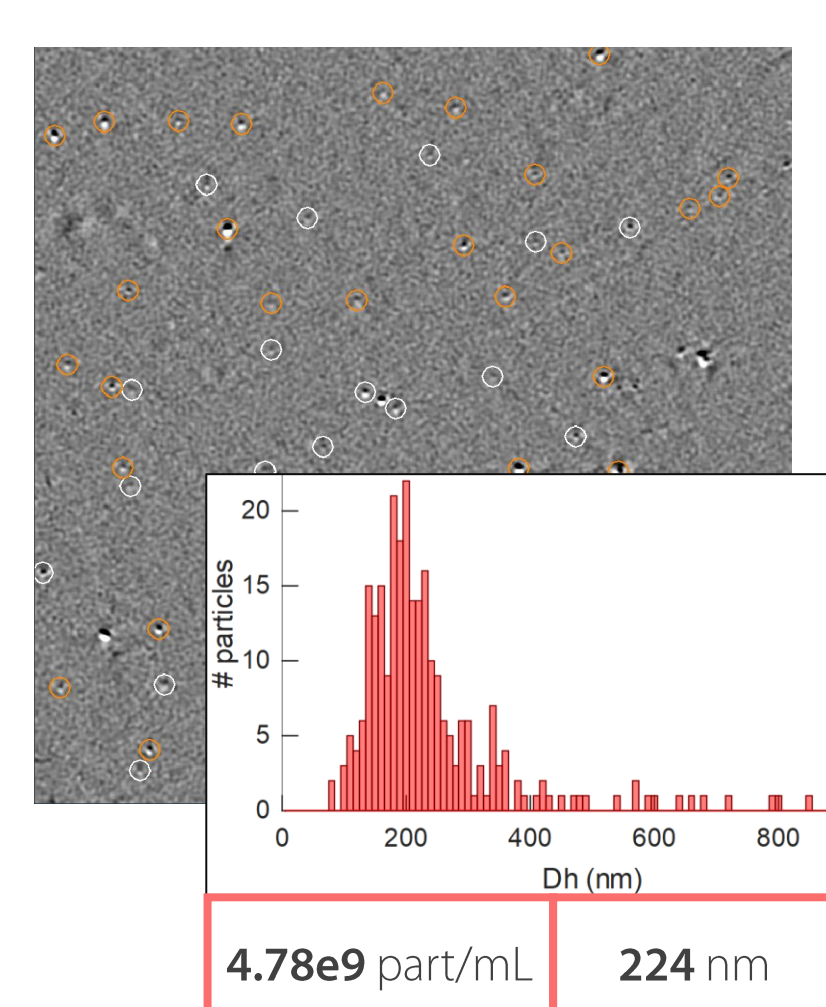
HYDRODYNAMIQUE DIAMETER



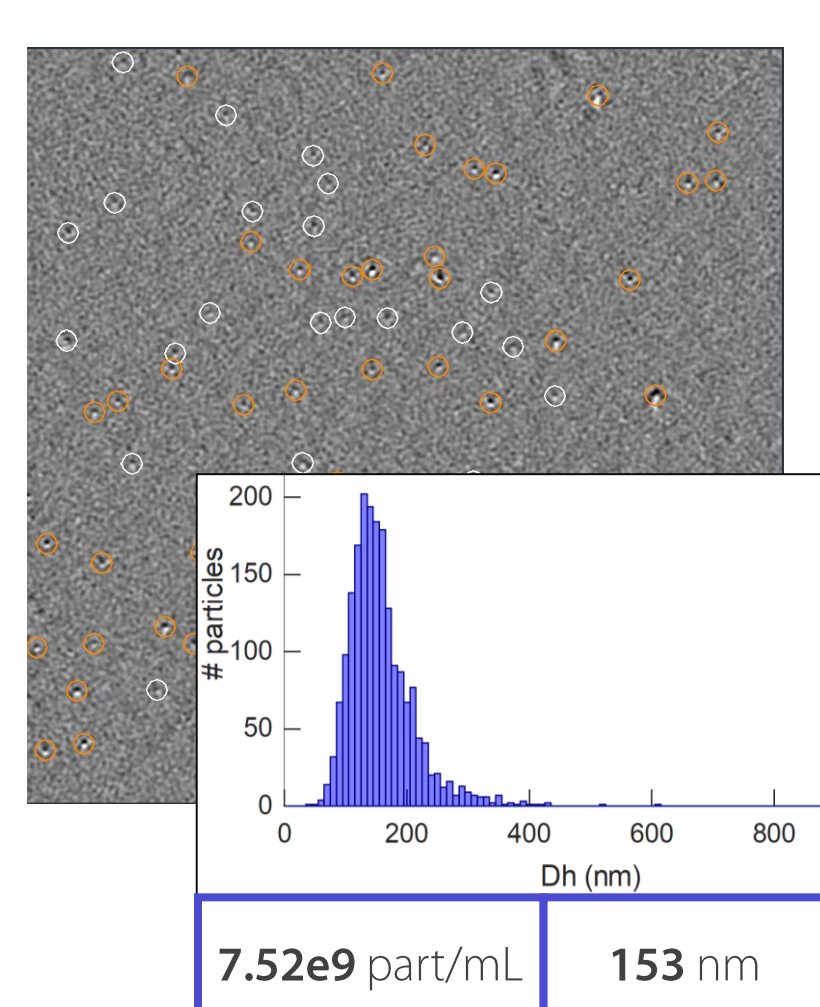
The comparison between NTA and ILM median diameter estimation shows a good correlation with a **R² at 0.83**. Both devices are following the same tendency when analyzing complex aggregated samples.

OTHER VIRUSES

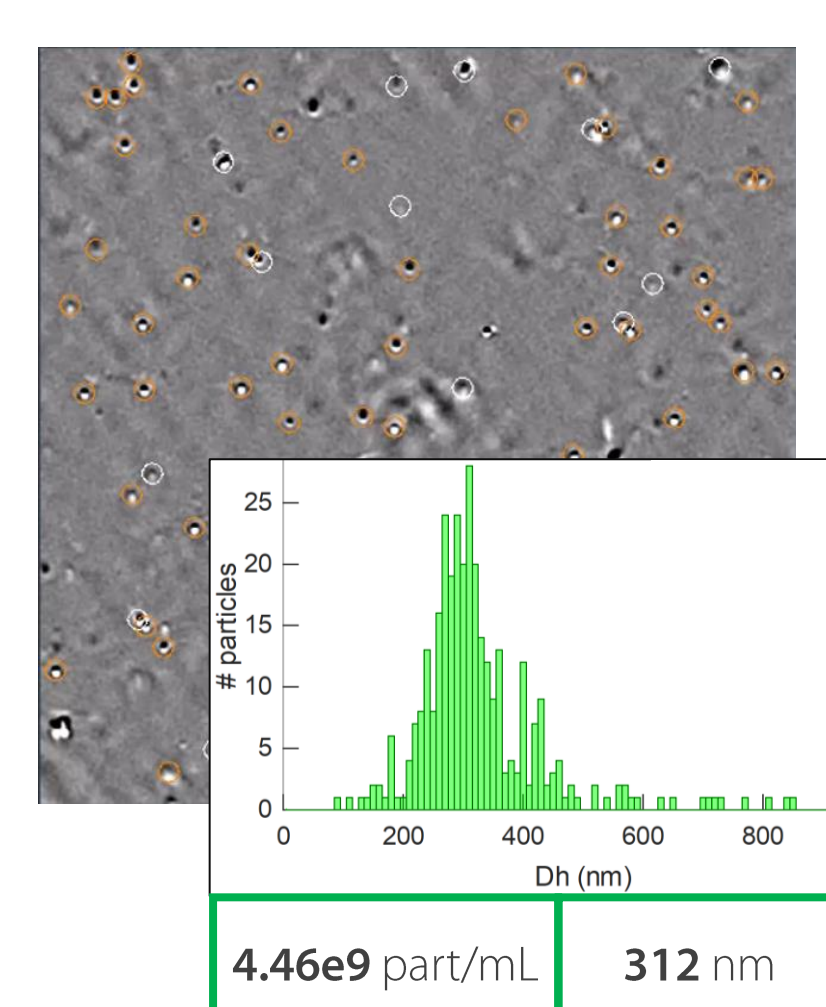
Baculovirus



HIV wt



Poxvirus



Videodrop was also tested on other viruses. Videodrop seems to be suitable for concentration and size measurement for diverse viral vectors (baculovirus, poxvirus), and infectious viruses (HIV)

Conclusion

- ✓ Strong correlation for lentivirus physical titer determination between Videodrop and 3 other methods (p24 ELISA, RTqPCR, NTA)
- ✓ Consistent size correlation for aggregated thermal stress-induced lentivirus between Videodrop and NTA
- ✓ Promising results on other viruses (Baculovirus, HIV and Poxvirus).

Videodrop appears suitable for viral vector quantification and characterization. It is an ideal companion for production process optimization and quality controls.

