PHAGES in LYON 2023

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RAPID CHARACTERISATION OF PHAGE SUSPENSIONS BY INTERFEROMETRY PART 2: TOWARDS IN PROCESS SECURISATION OF PURIFICATION

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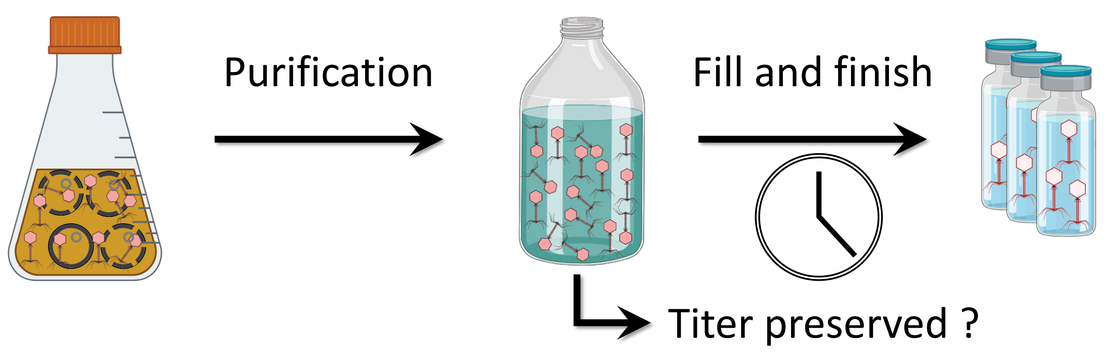
INTRODUCTION



Phage production's downstream process includes a critical purification step susceptible to destabilise phage suspensions. Ideally, phage titer should be determined before fill and finish to avoid production losses, but titration is a time-consuming method. The colloidal properties of phages can be used to rapidly determine the concentration and size distribution of suspended particles, e.g., with interferometric light microscopy (ILM) method.

Can ILM successfully monitor phage purification ?

MATERIALS AND METHODS

Phage purification

1 **Frontal filtration**

Purification intermediate (PI)

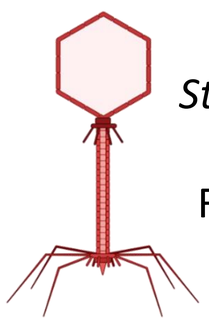
2 **Tangential flow filtration**

Diluted PI (dPI)

Washed PI (wPI)

Concentrated PI (cPI)

Formulated PI (fPI)

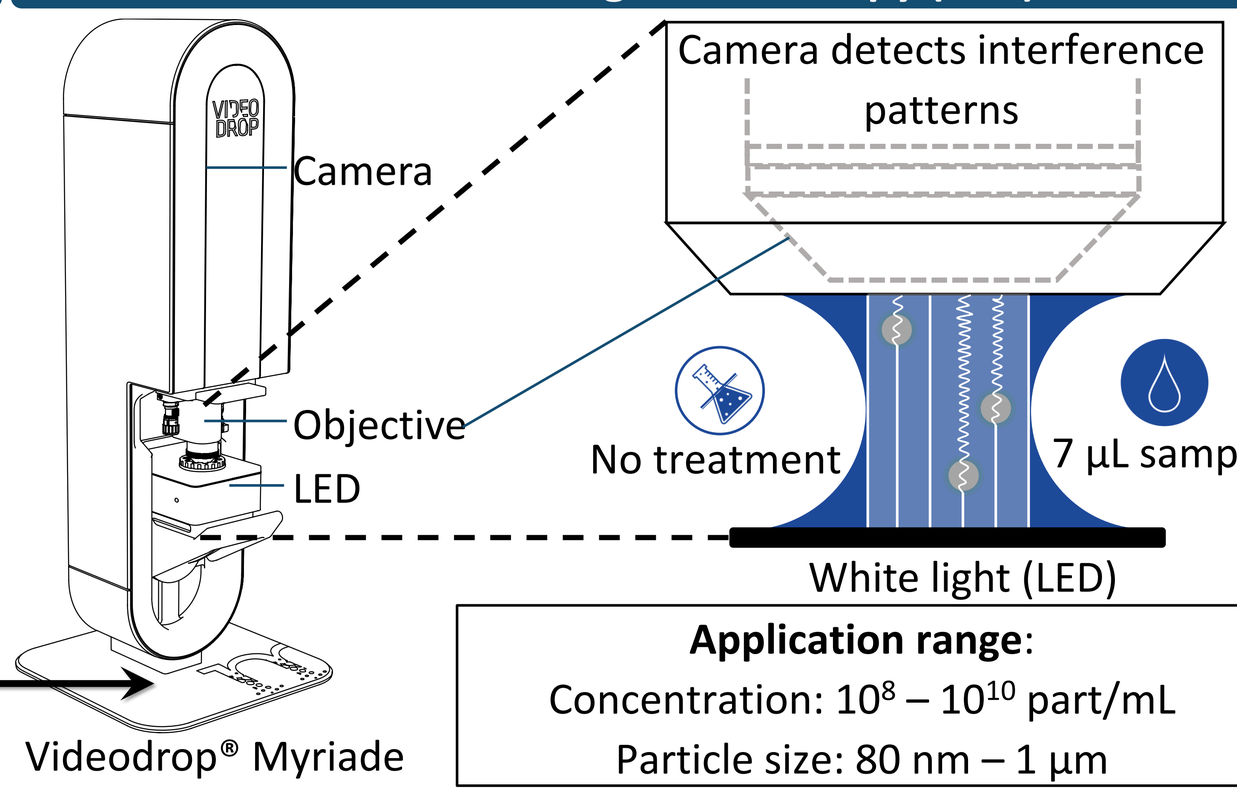


Phage anti *Staphylococcus aureus*
vB_SauM-V1SA20
Family: *Herelleviridae*
Genus: *Silviavirus*
Myovirus

N=3 purifications

50 µL sampled at each steps:
- Phage titer (N=3 spot –test measures)
- Particle concentration/titer and size (N=2 ILM measures)

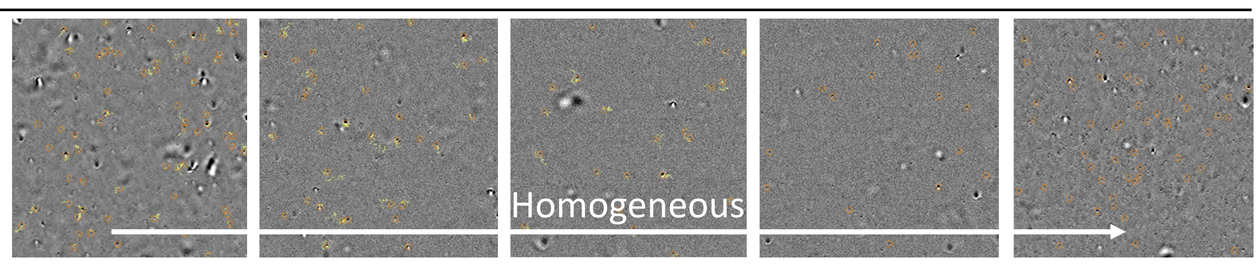
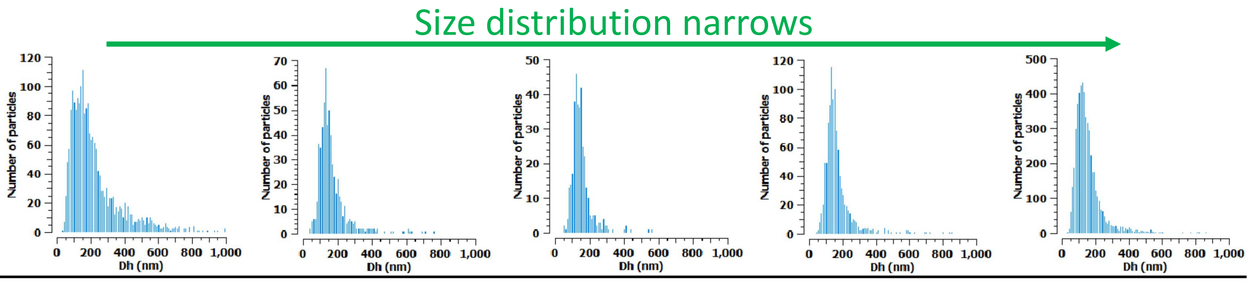
Interferometric light microscopy (ILM)

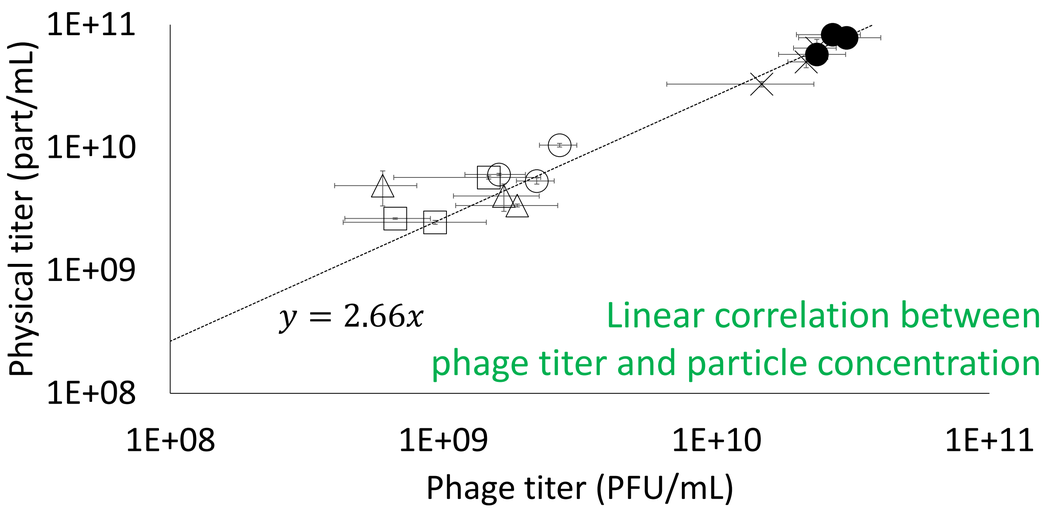


Application range:
Concentration: 10^8 – 10^{10} part/mL
Particle size: 80 nm – 1 µm

RESULTS

Example of purification n°2

Steps	PI	dPI	wPI	cPI	fPI
ILM image					
Phage titer (PFU/mL)	(2.3 ± 0.6)E10	(1.6 ± 0.4)E9	(6.7 ± 2.3)E8	(1.7 ± 0.6)E9	(2.1 ± 0.3)E10
	Phage titer preserved →				
Physical titer (part/mL)	(5.7 ± 0.5)E10	(6.0 ± 0.1)E9	(2.6 ± <0.1)E9	(4.0 ± 1.0)E9	(5.0 ± 0.5)E10
Mean particle diameter (nm)	175 ± 8	171 ± 3	160 ± 8	166 ± 1	152 ± 3
	Size distribution narrows →				
Size distribution diagram					



$$Deviation = (\log(particle\ concentration) - \log(titer))/\log(titer)$$

Deviation	PI	dPI	wPI	cPI	fPI
Purification 1	5 %	4 %	5 %	3 %	3 %
Purification 2	4 %	6 %	7 %	4 %	4 %
Purification 3	4 %	6 %	6 %	10 %	4 %
Mean ± SD	(4 ± 1) %	(6 ± 1) %	(6 ± 1) %	(6 ± 4) %	(4 ± <1) %

CONCLUSION

Assets:

Small sample (5-10 µL)
No necessary treatment
At any viscosity
Rapid measure (a few minutes)

Monitoring of purification:
- ✓ Does the suspension become homogeneous?
- ✓ Is the physical titer preserved?
- ✓ Does the size distribution narrow?

Linear correlation ⇨ physical titer may estimate phage titer

Limits:

Highly concentrated samples (**>10^8 part/mL**)
The higher the concentration, the more precise the result (lowest deviation of 4 % reached for PI and fPI)
Detects particle > 80 nm ⇨ **not for podovirus** morphotype
Measures particles, not phages:
⇨ **Cannot detect phages with emptied capsid**
⇨ **Cannot replace titration as release quality control**

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