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Transmission electron microscopy

Utilising a powerful tool in biosafety as a novel approach to characterise the product quality of biologics such as vector-based vaccines and gene therapy products

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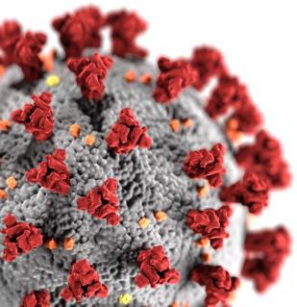

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Outline

1. Transmission electron microscopy: A short introduction
2. TEM in Biotechnology: Viral safety of biologics
3. From viral safety to product characterisation
4. Sample morphology, distribution & purity
5. Particle quantity: semi-quantification using TEM
6. Summary

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1. Transmission electron microscopy: A short introduction

Fig. 1 Transmission electron microscope

- Electron acceleration up to 300kV
- Electron interaction with stained sample as electrons transmit sample
- Image acquisition (analogue or digital)
- Resolving power below 1nm
- Deployed in medicine, bioscience and materials science for ultrastructural analysis

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1.2 Transmission electron microscopy: A short introduction

Sample preparation in bioscience for TEM

I. Cellular diagnostics via positive staining *(psTEM, sample is stained)*

steps: chemical fixation → resin embedding → ultrathin sectioning → staining

II. Particle diagnostics via negative staining (nsTEM, background is stained, particles appear bright)

steps: sample deposition and sedimentation on TEM grid → staining

Staining agents

- Uranyl acetate (UA)
- Lead citrate
- Phosphotungstic acid (PTA)

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2. TEM in Biotechnology: Viral safety of biologics

ICH Guidelines:

ICH Q5A (R1) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

Where is TEM utilised?

1. Cell bank characterisation (psTEM)
2. Bulk harvest screening (nsTEM)

Quality Assurance?

- Qualified and validated assays following **GMP**

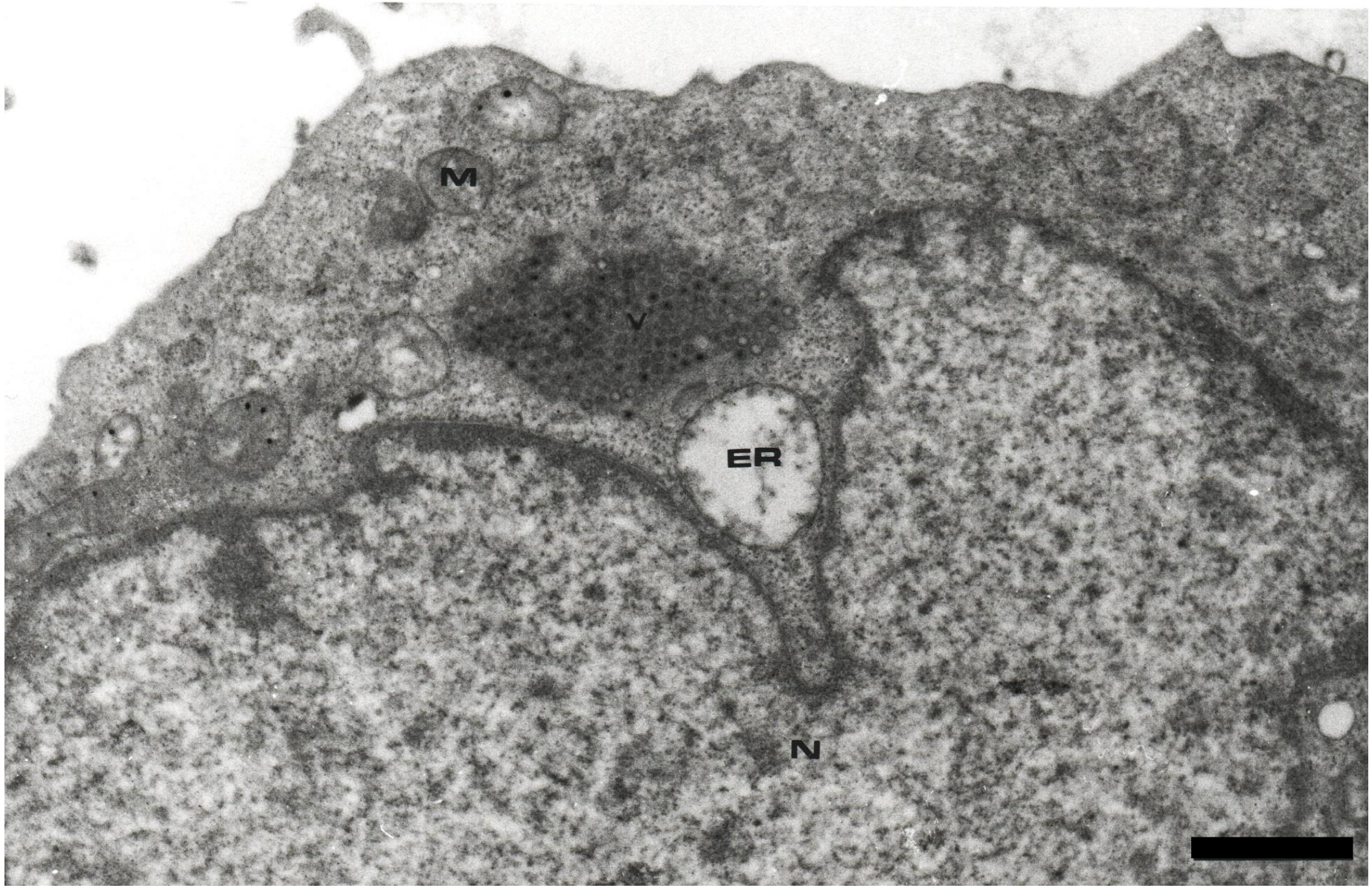

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Fig. 2 Ultrathin section of mammalian cells with intracellular aggregation of virus particles (**V**). (scale bar = **1µm**)

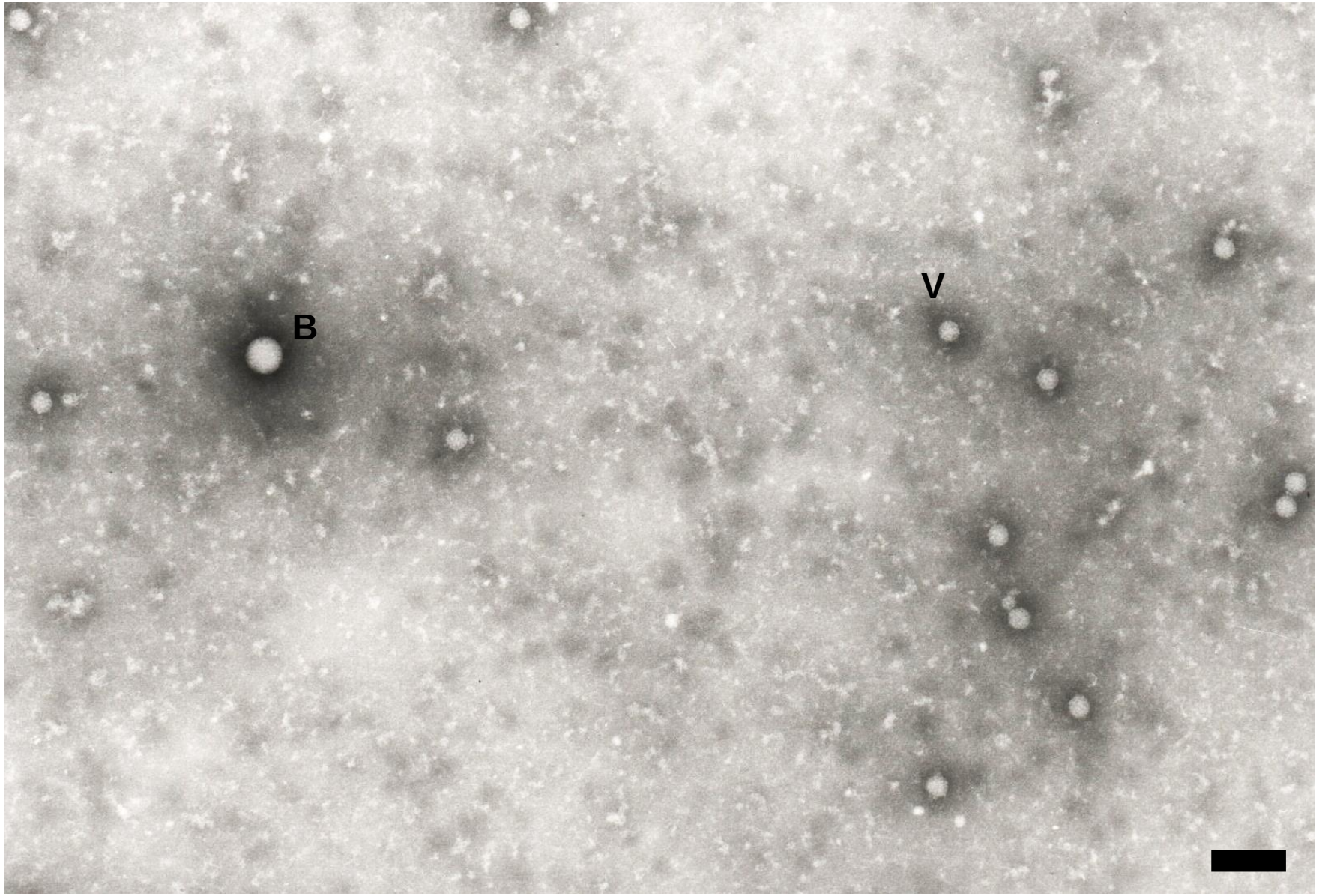

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Fig. 3 nsTEM image of virus like particles (**V**) and latex beads (**B**). (scale bar = **200 nm**)

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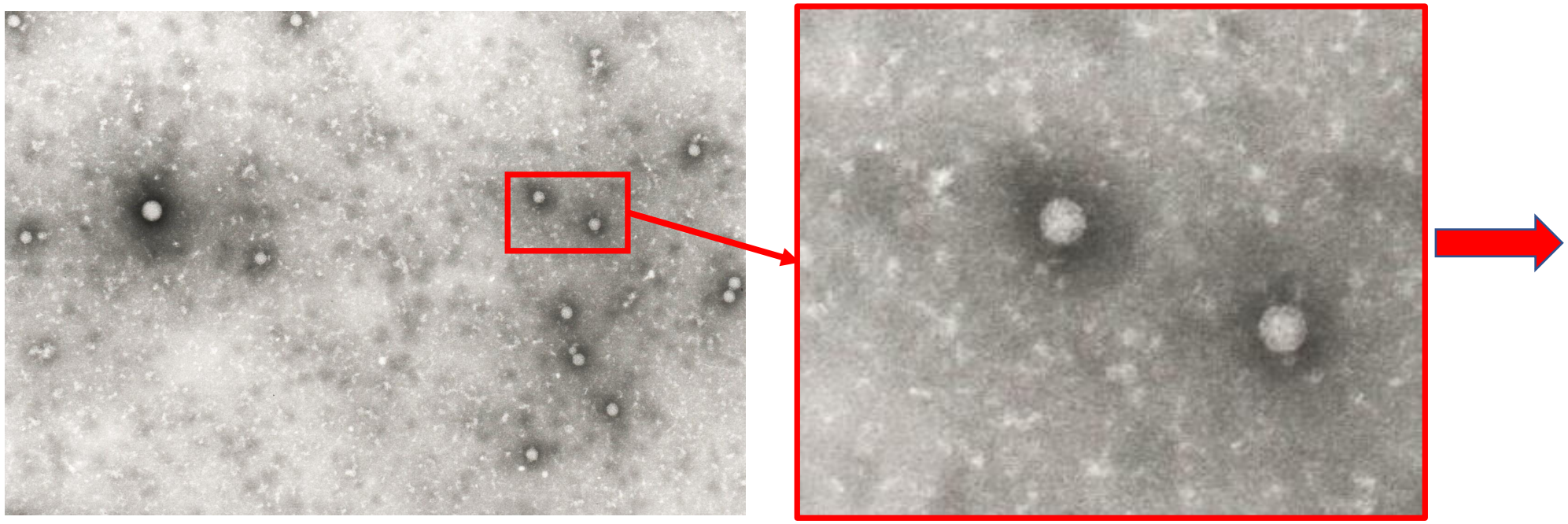
3. From viral safety to product characterisation



- **Morphology**
- **Distribution**
- **Purity**
- **Semi-quantity**

- **Detection limit** for TEM analysis: 10^8 - 10^9 particles per mL

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4. Sample morphology, distribution & purity

What can TEM tell me about my sample?

1. **Particle morphology:**
   - **Virus structure**
     - **Identity: enveloped/non-enveloped, capsid structure**
     - **Structural integrity: damaged or intact particles**
2. **Particle distribution:**
   - **Aggregation of particles (unsuitable process conditions in USP or DSP?)**
3. **Sample purity:**
   - **Background noise (cell debris, high protein contamination)**

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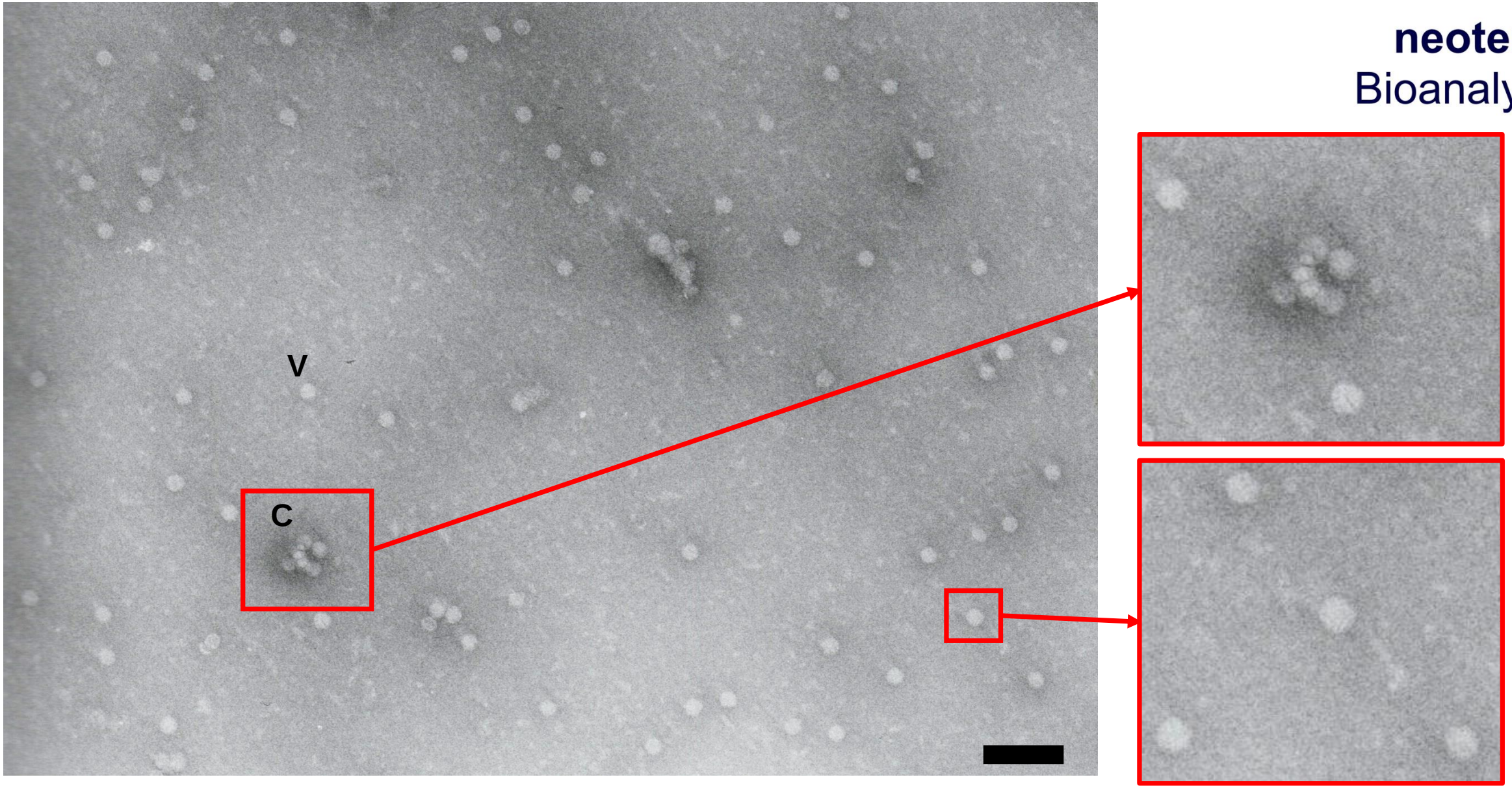

Fig. 4 nsTEM image of ***Adeno-associated viruses*** (**V**) & particle clusters/aggregations (**C**). (scale bar = **100 nm**)

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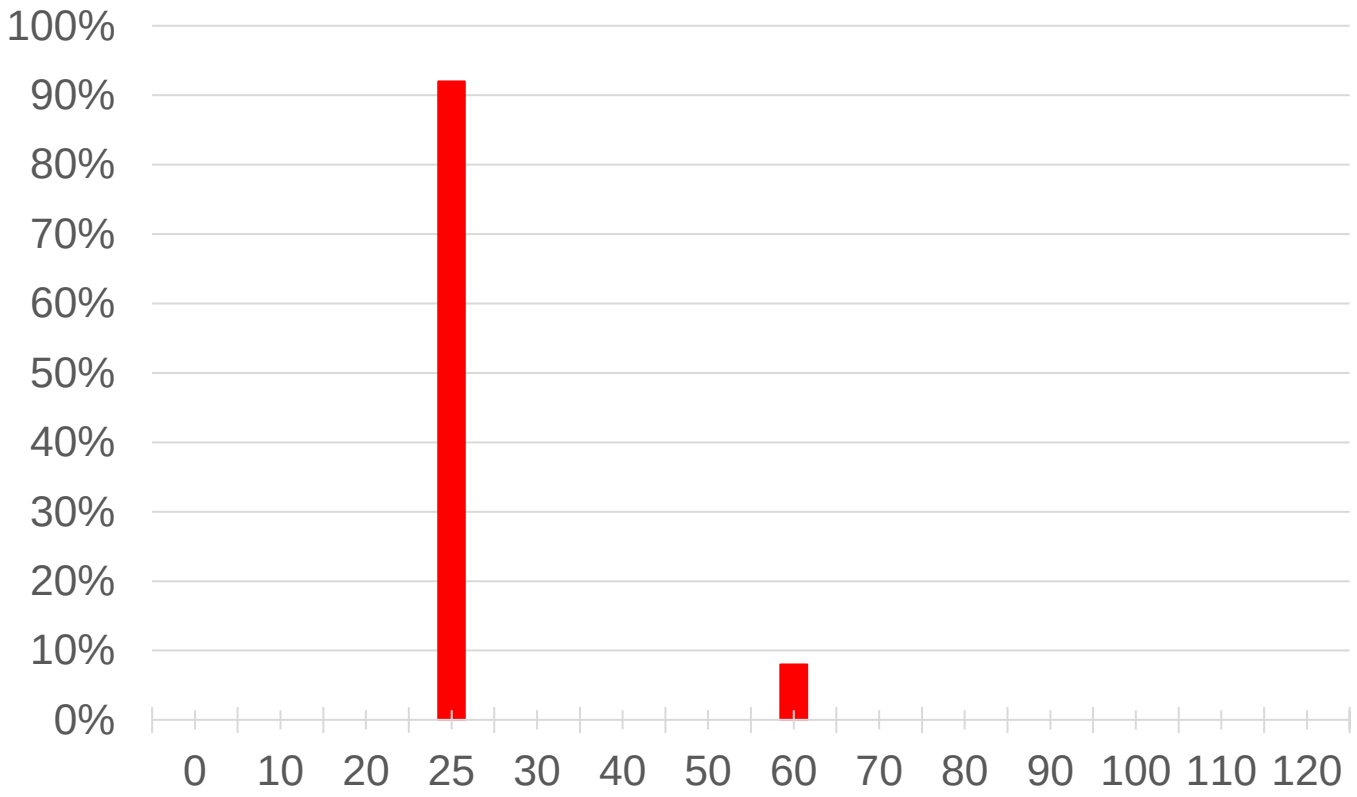
4. Sample morphology, distribution & purity

Percentage of counted particles



Average particle size in **nm**

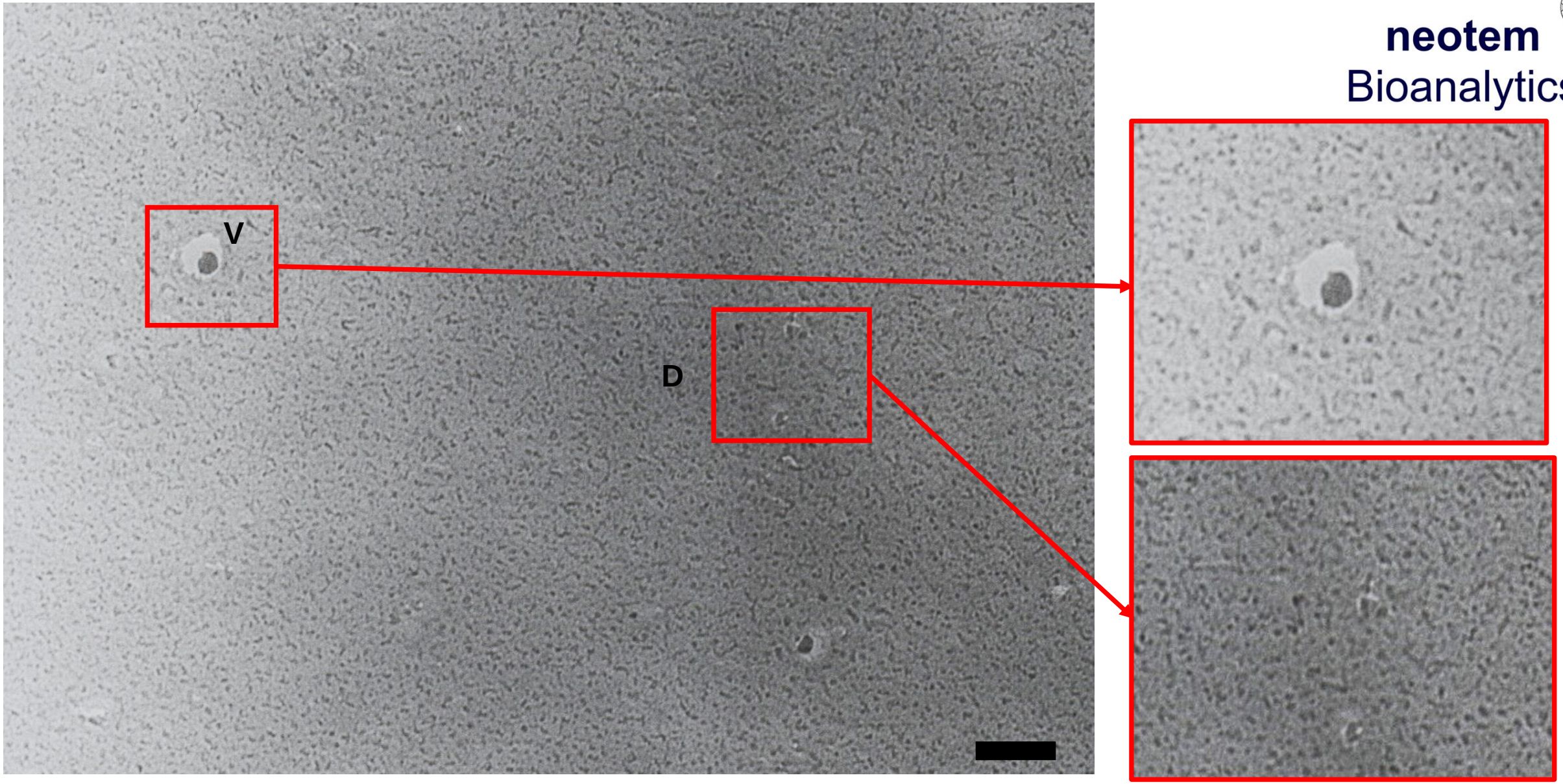


Fig. 5 nsTEM image of damaged Adeno-associated viruses (**V**) & sample debris (**D**). (scale bar = **100 nm**)

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5. Particle quantity: semi-quantification using TEM

Procedure

- A suspension of beads with a known concentration and size are added to the test item
- Visualisation of test item and beads via nsTEM (negative staining)
- A defined amount of beads are counted on the TEM grid
- Test item particles are counted parallel to the beads
- Calculation of test item concentration in relation to the beads concentration

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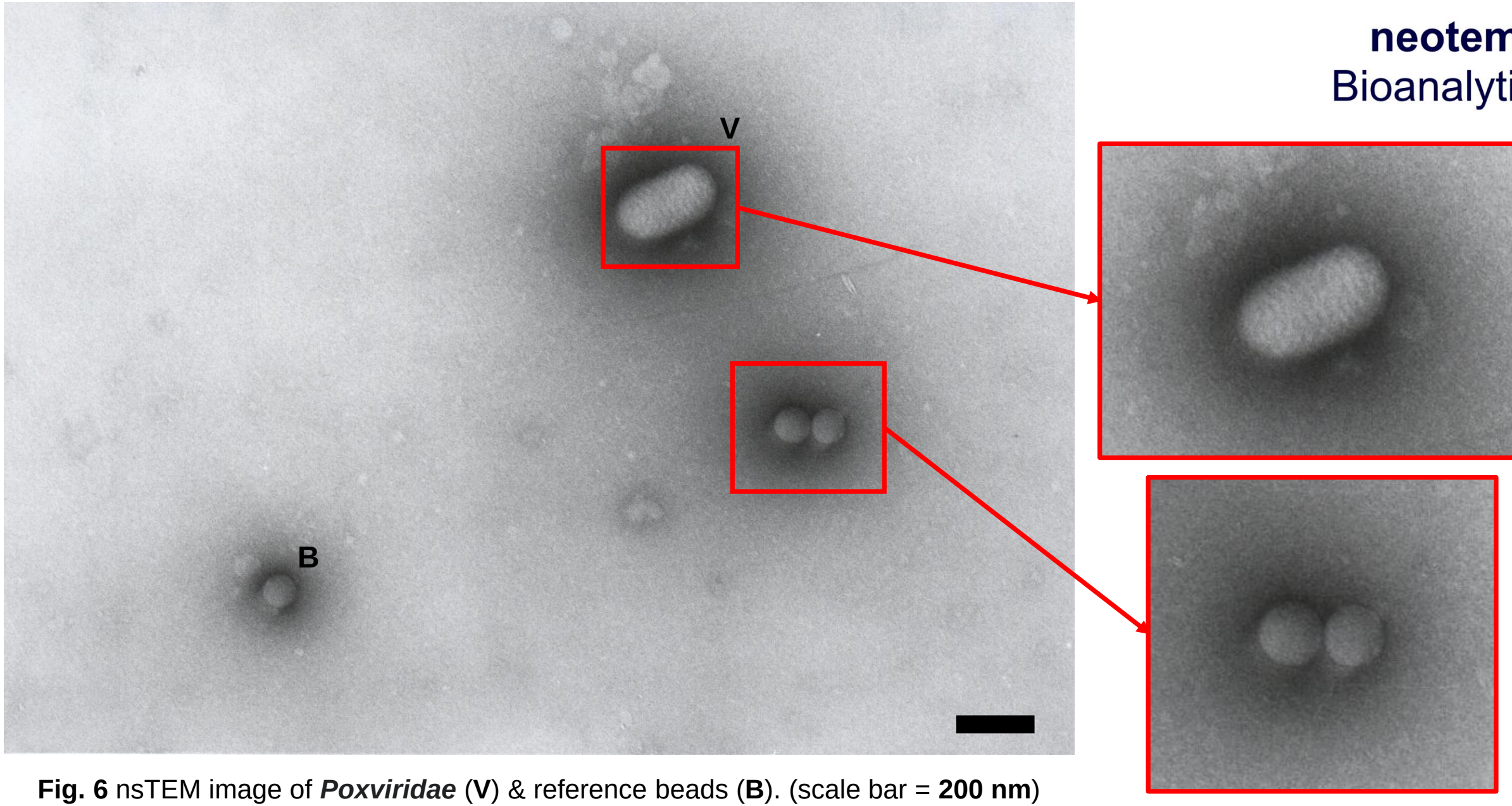

Fig. 6 nsTEM image of ***Poxviridae*** (**V**) & reference beads (**B**). (scale bar = **200 nm**)

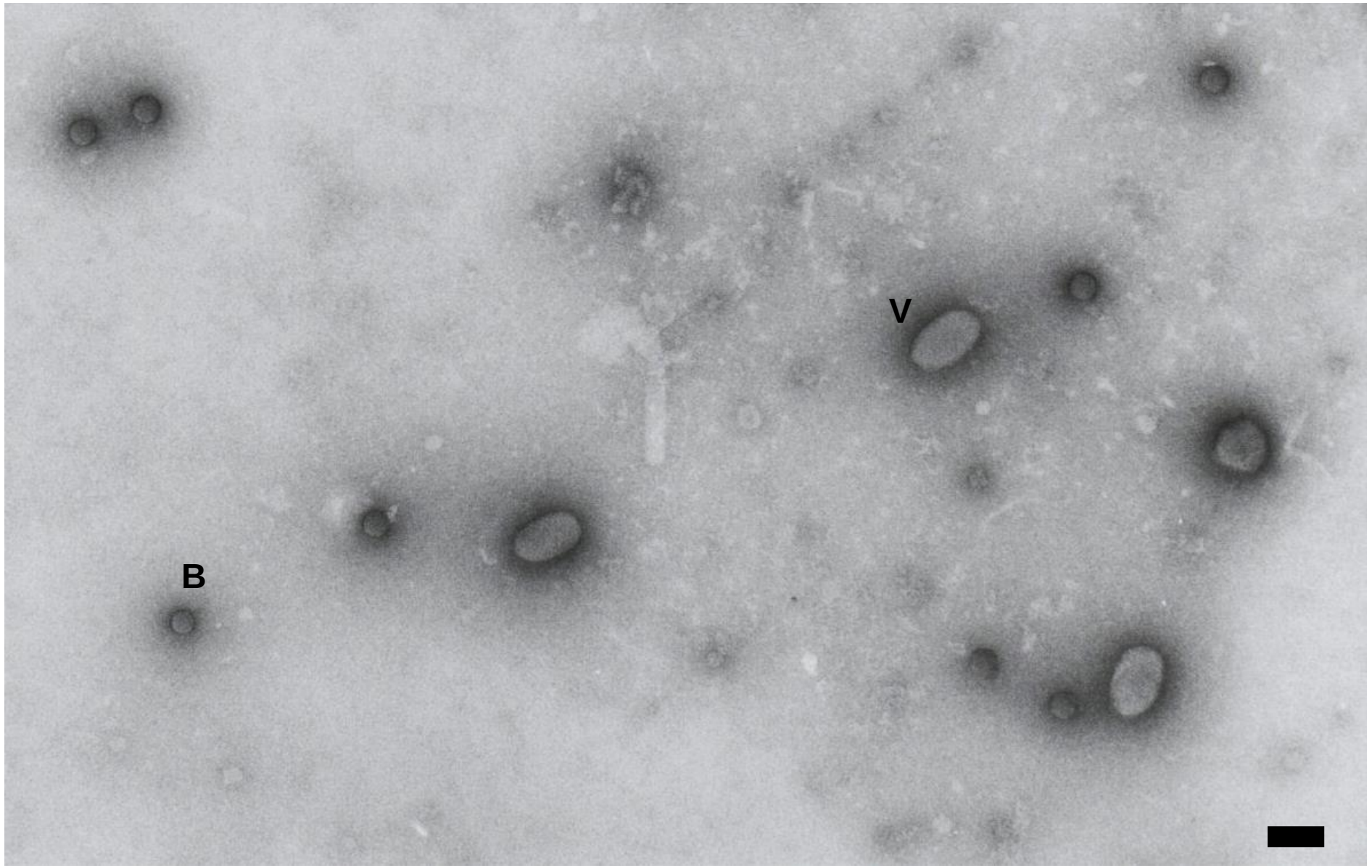

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Fig. 7 nsTEM image of ***Poxviridae*** (**V**) & reference beads (**B**). (scale bar = **200 nm**)

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6. Summary

- **TEM can deliver visual feedback on particle:**
  - **morphology & structure/integrity**
  - **distribution & aggregation**
  - **purity**
  - **semi-quantity**
- **TEM can visualise the ultrastructural properties of producer cells**
  - **Information which can improve process conditions for vector-based vaccines and gene therapeutics in USP & DSP**

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TEM expertise for powerful sample analysis

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