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Exploring the Invisible: Electron microscopy's role in safeguarding viral safety and advancing gene therapy

3rd ViruSure Workshop
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Vienna

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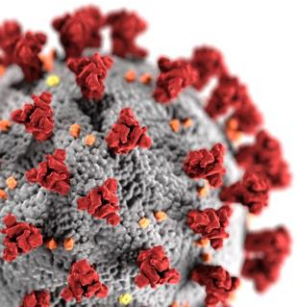

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1 Company Background

- Formally known as the ZUD (Zentrum für Ultrastrukturelle Diagnostik)
- Founded 1999 by Dr. Dietrich Bäumer & Dr. Uwe Kahmann in Bielefeld
- Part of the IIT-GmbH (Institut für Innovationstransfer an der Universität Bielefeld GmbH)
- Location: Campus Bielefeld University, Germany
- Team of five employees
- **GMP certified**

Fig. 1. Campus Bielefeld & company location

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2 Portfolio & Equipment

EM-diagnostics for the biopharmaceutical industry

1. Cell bank characterisation (ICH Q5A)
2. Bulk harvest screening (ICH Q5A)
3. Virus & vector analysis (Gene therapy products)

GMP TEMs

- Hitachi H500
- LVEM 25 Delong

3 Transmission electron microscopy: A short introduction

Fig. 2 Transmission electron microscope

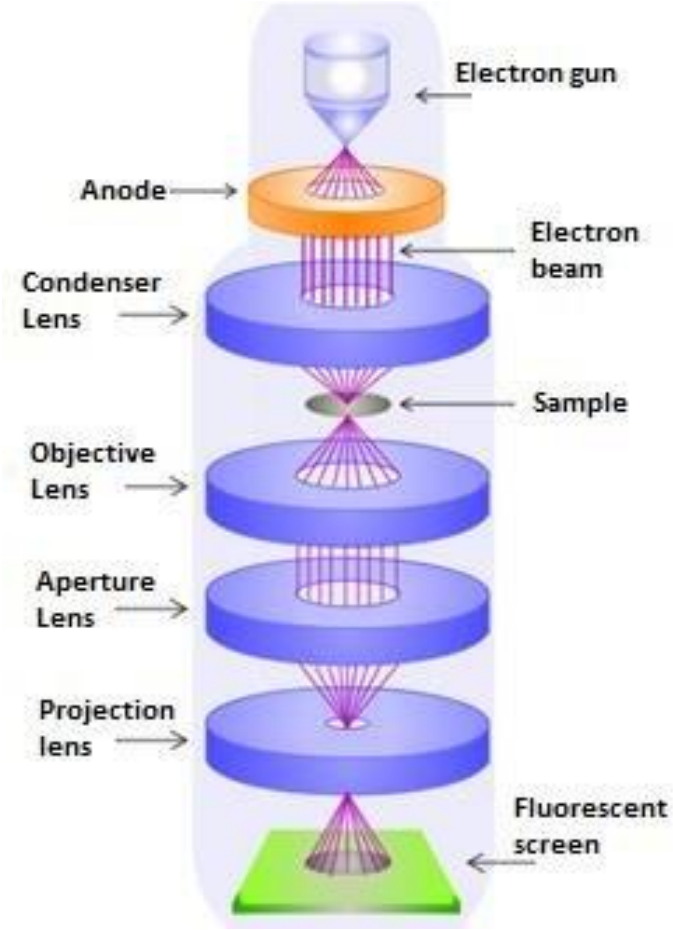

Fig. 3 TEM principle [1]

4 TEM in Biotechnology

ICH Guidelines:

ICH Q5A (R2) Quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin

Viral safety: Adventitious agents

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

Virus & vector characterisation

3. AAV (full-empty ratio, aggregation, purity & integrity) (**nsTEM**, **no-stainTEM** **&** **CryoTEM**)
4. Nanoparticles (**nsTEM** & **CryoTEM**)

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5 Transmission electron microscopy: Sample preparation & imaging

Positive staining TEM

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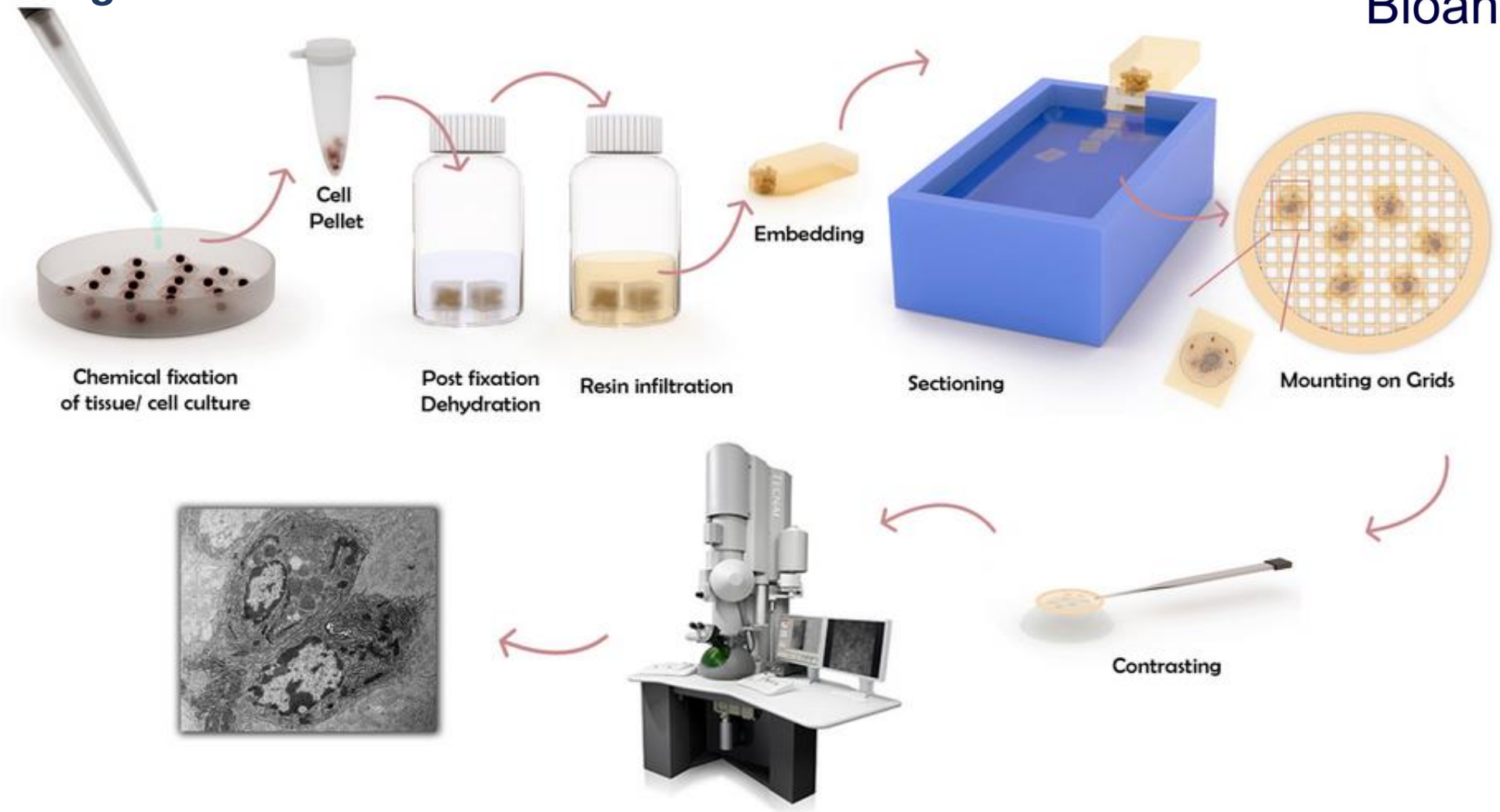

Fig. 4 An illustration of the chemical fixation method (Figure By Neta Varsano, Weizmann Institute of Science)

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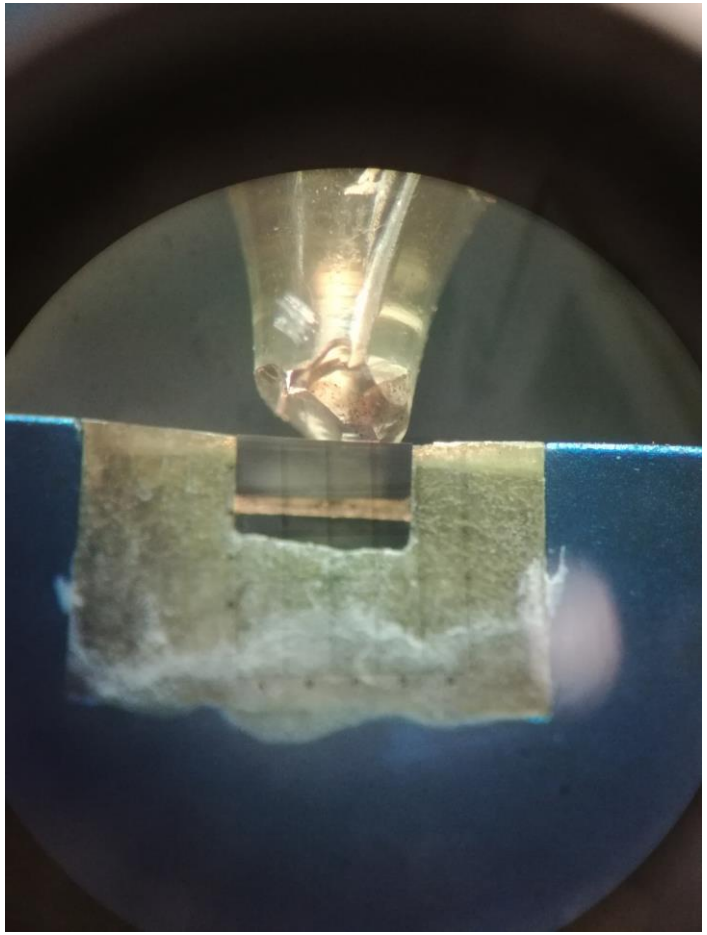

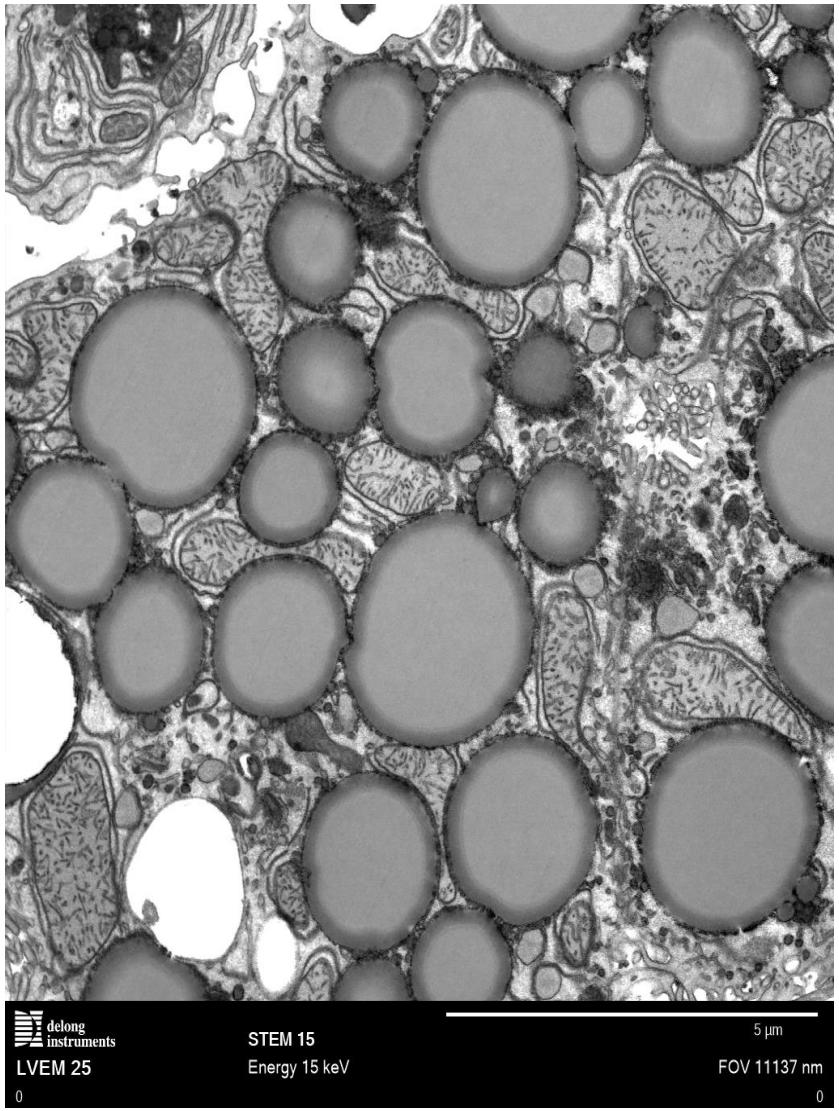

Fig. 5 Ultrathin sectioning

Fig. 6 psTEM image of liver cells

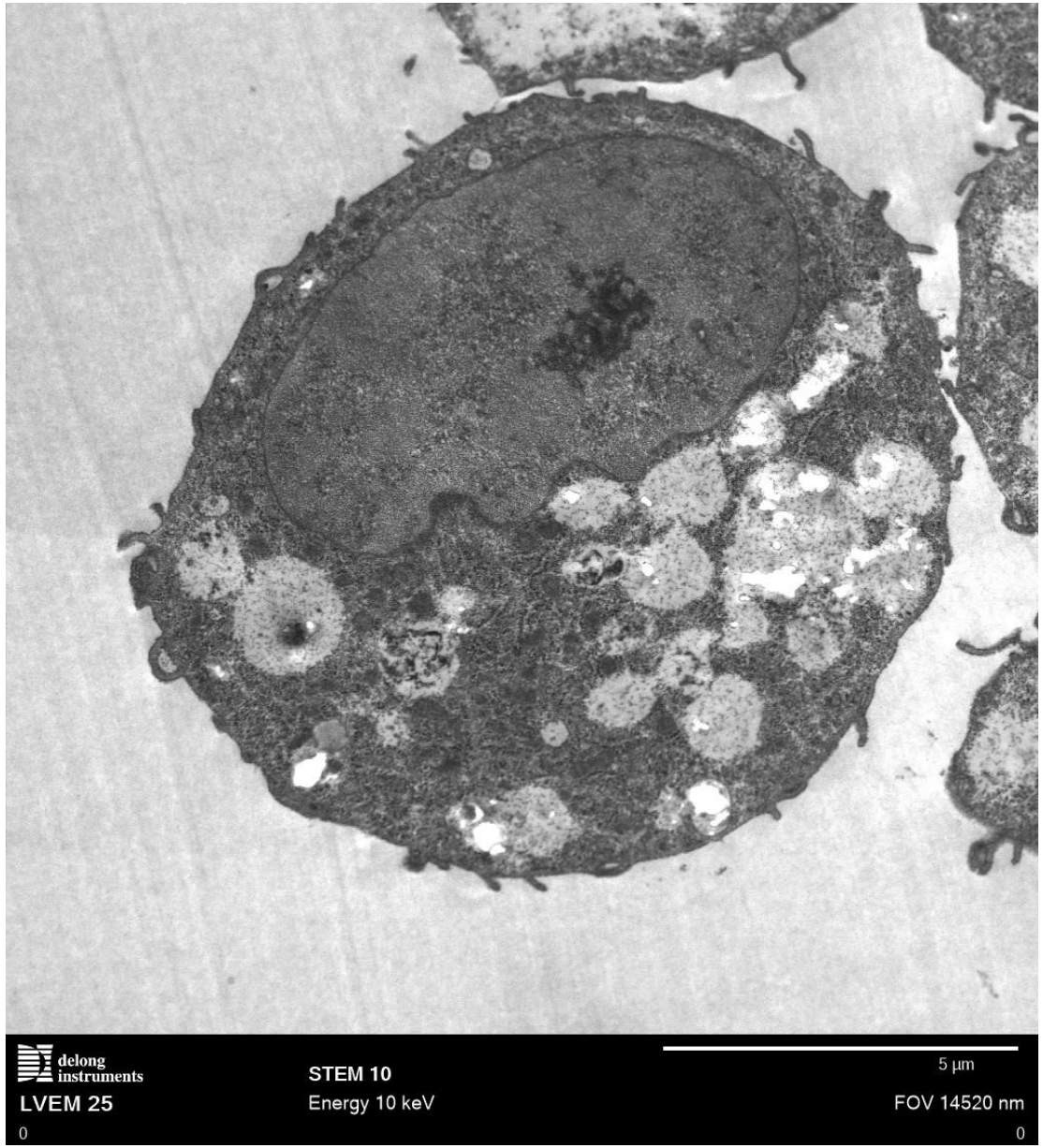

Fig. 7 psTEM image of CHO cells (LVEM 25)

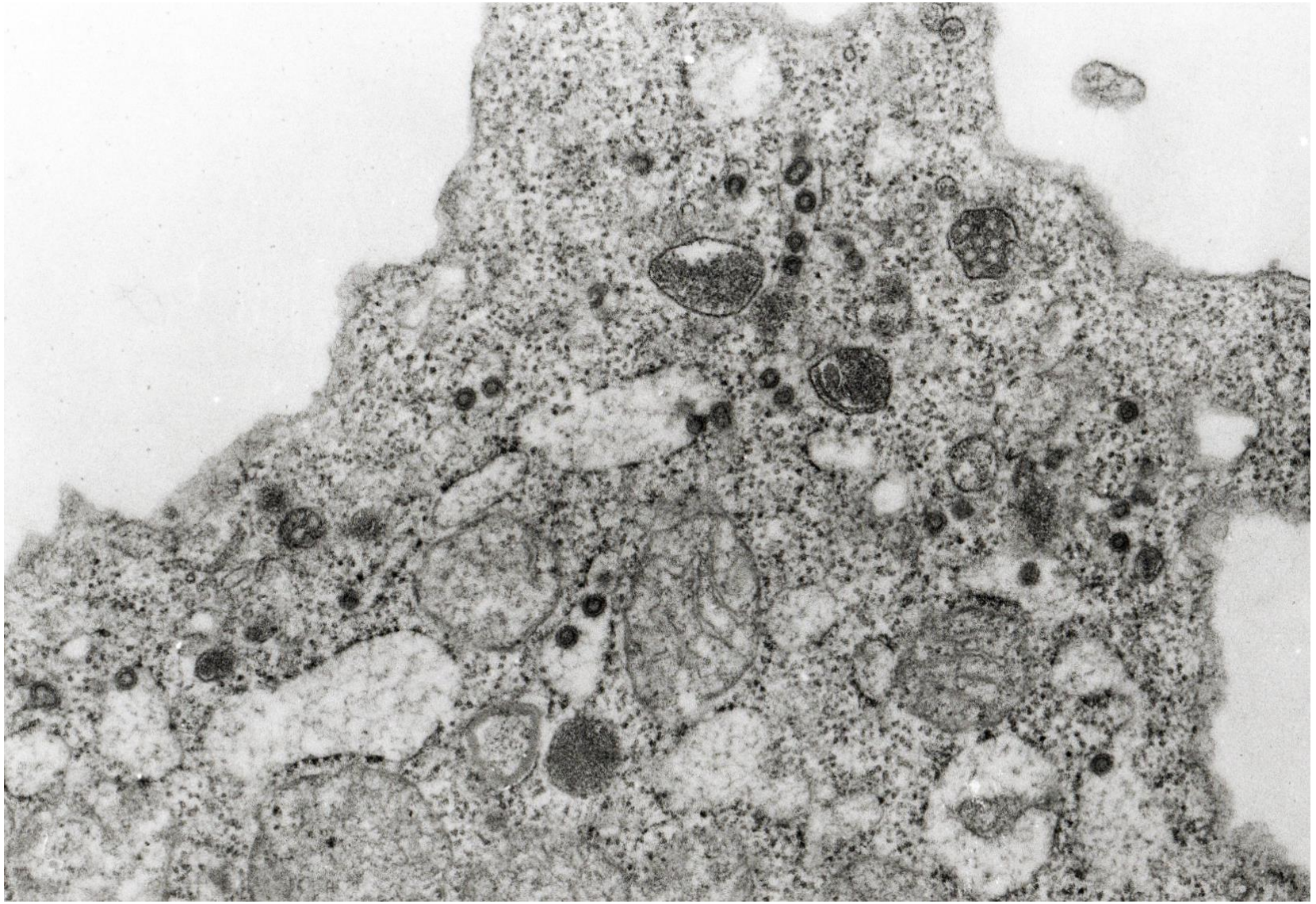

Fig. 8 psTEM image of intracisternal RVLPs (Hitachi H500)

Negative staining TEM

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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)
- Phosphotungstic acid (PTA)

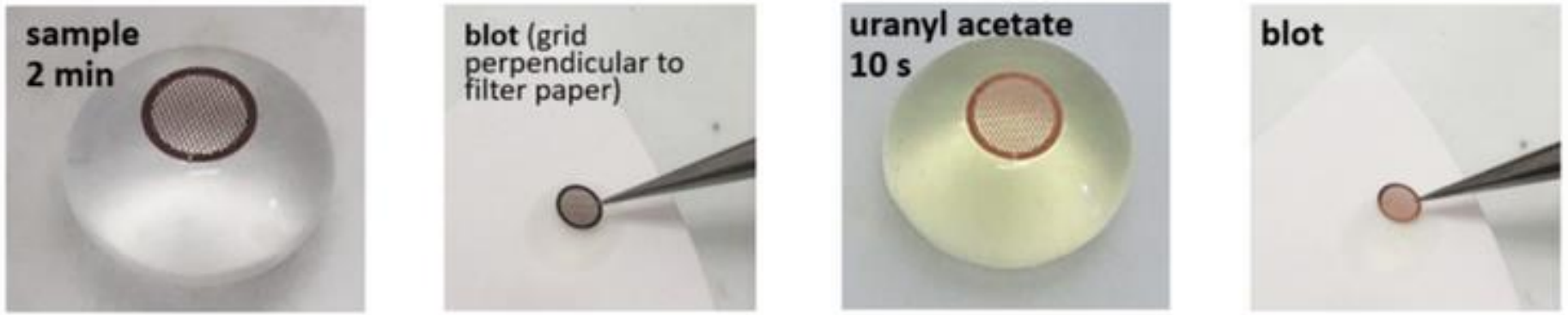

Fig. 9 nsTEM sample preparation [3]

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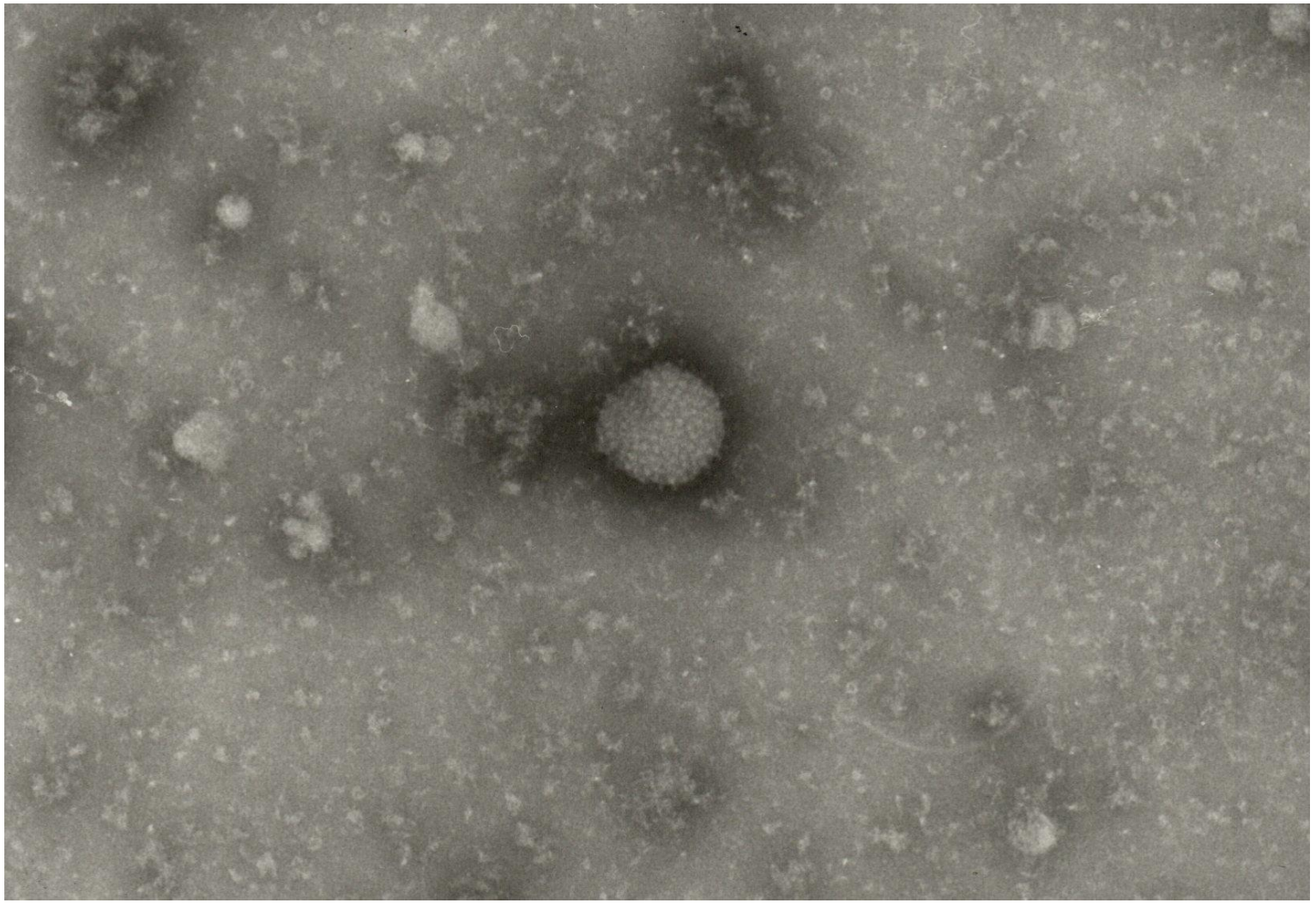

Fig. 10 nsTEM image of RVLP (Hitachi H500)

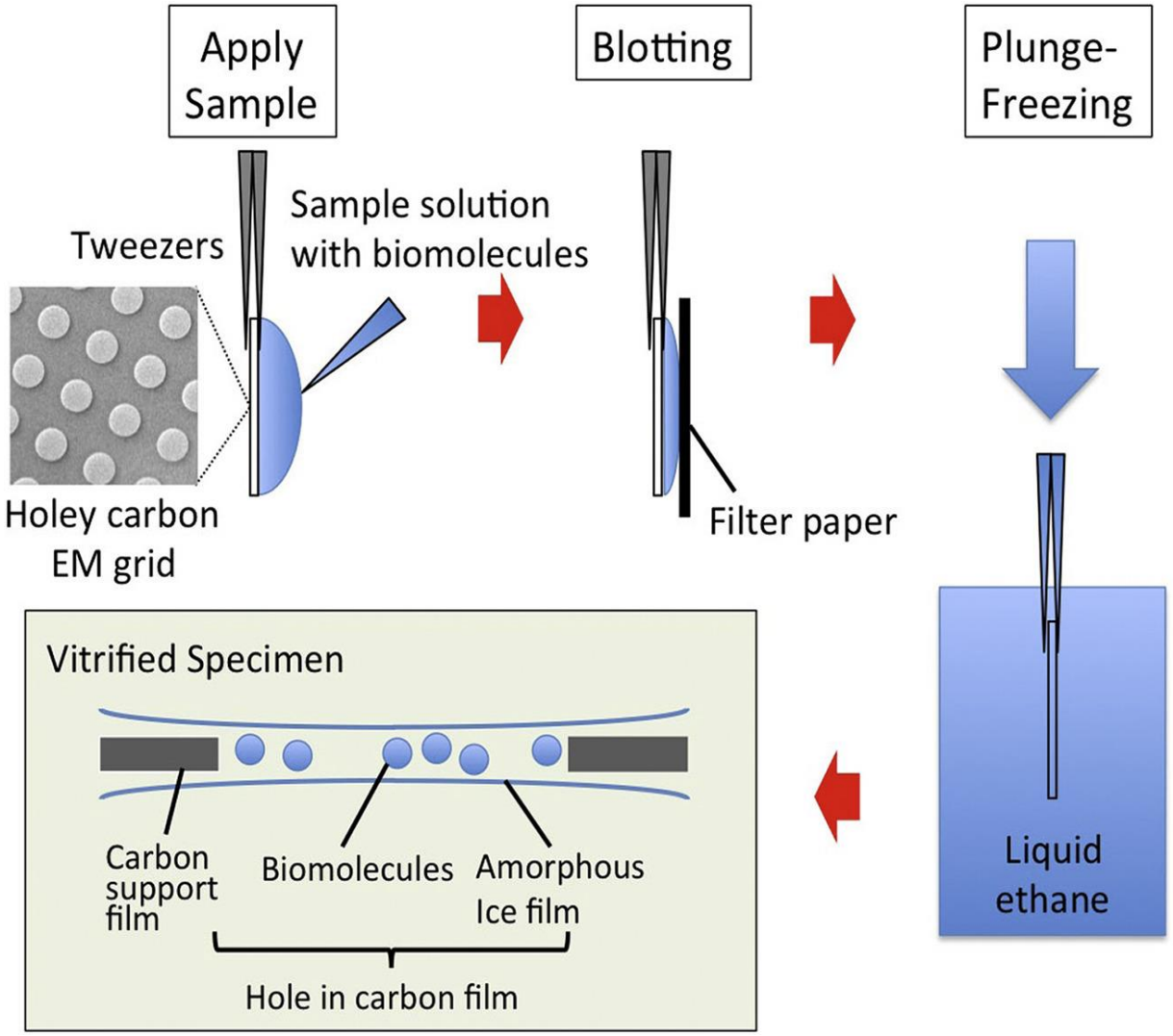

Fig. 11 CryoTEM sample preparation [2]

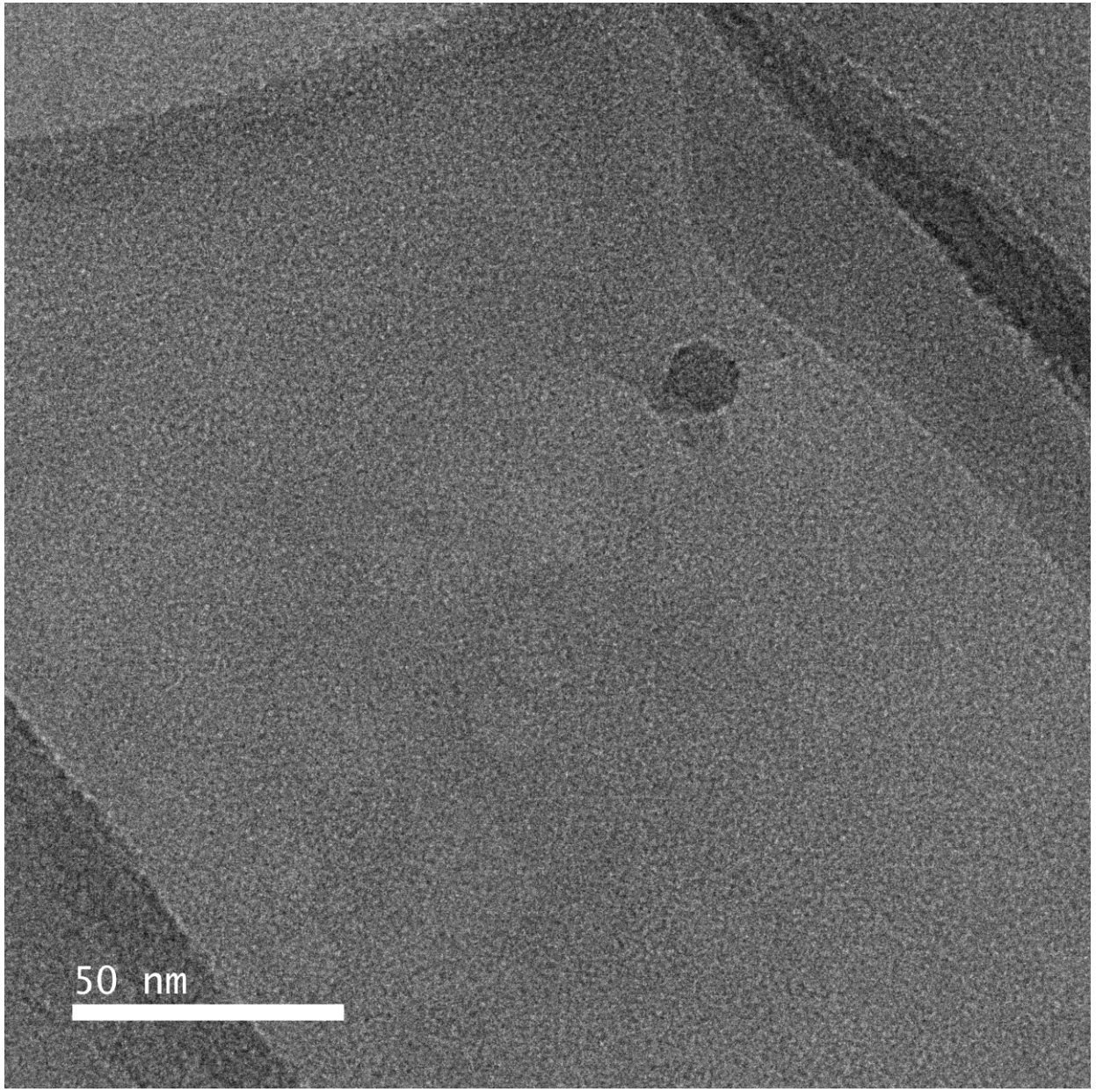

Fig. 12 CyroTEM image of AAV particle (JEM-2200FS, 200 kV)

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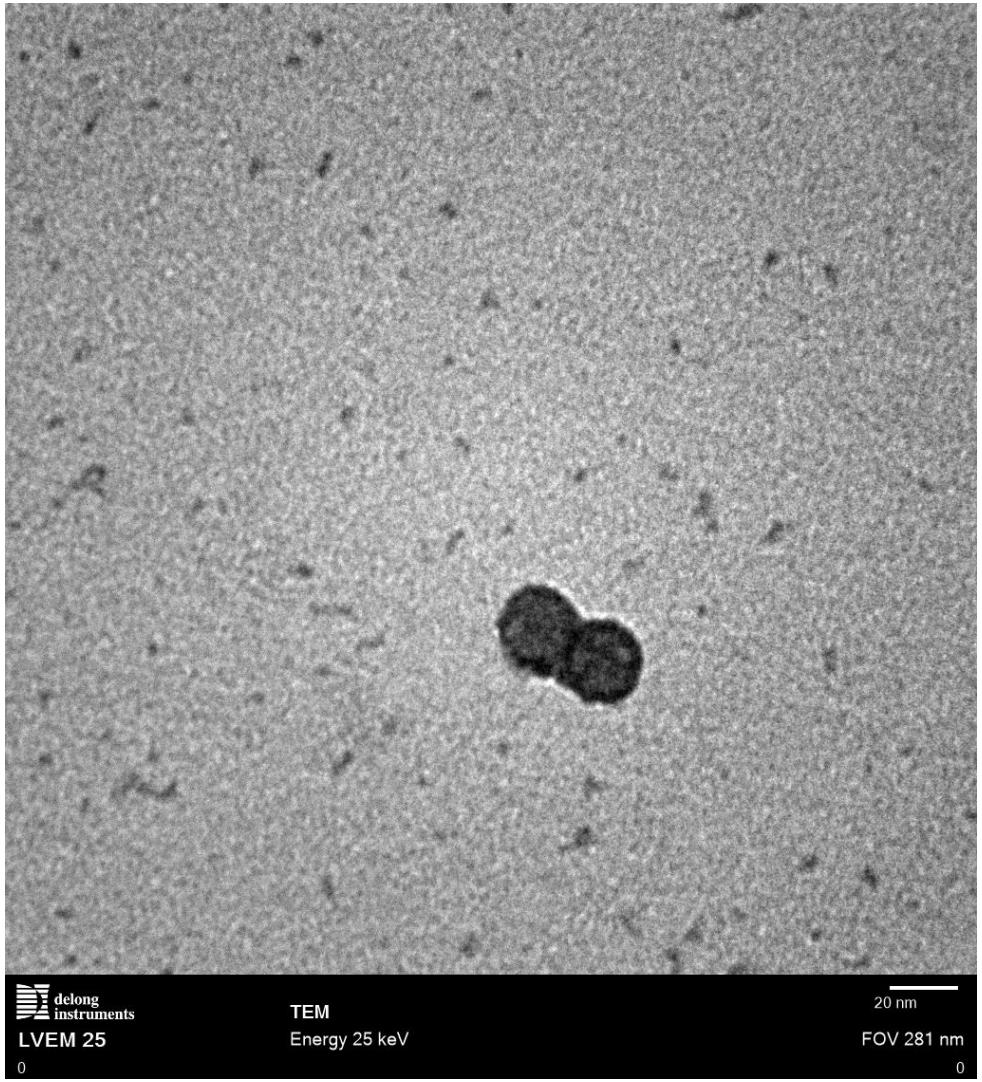

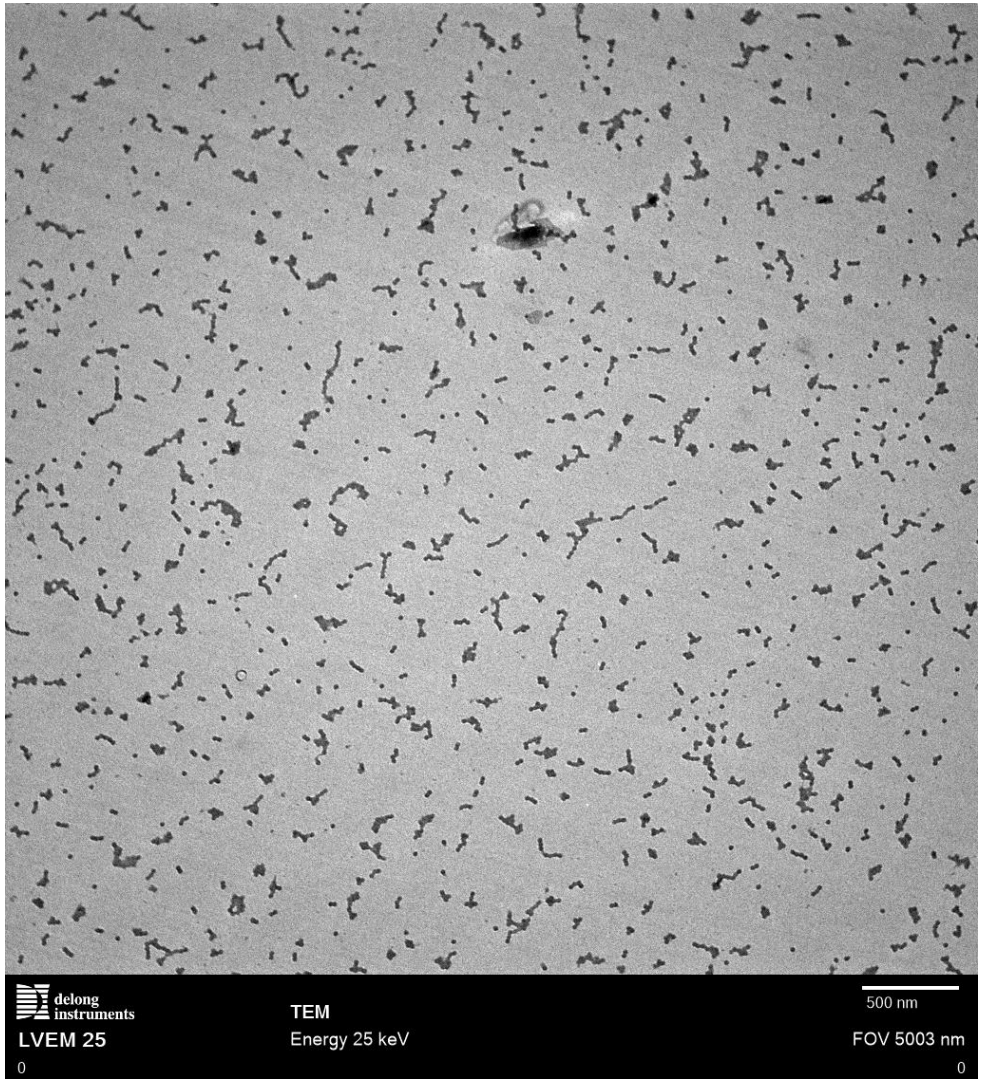

Fig. 13 & 14 no-stainTEM image of AAV particles (LVEM 25)

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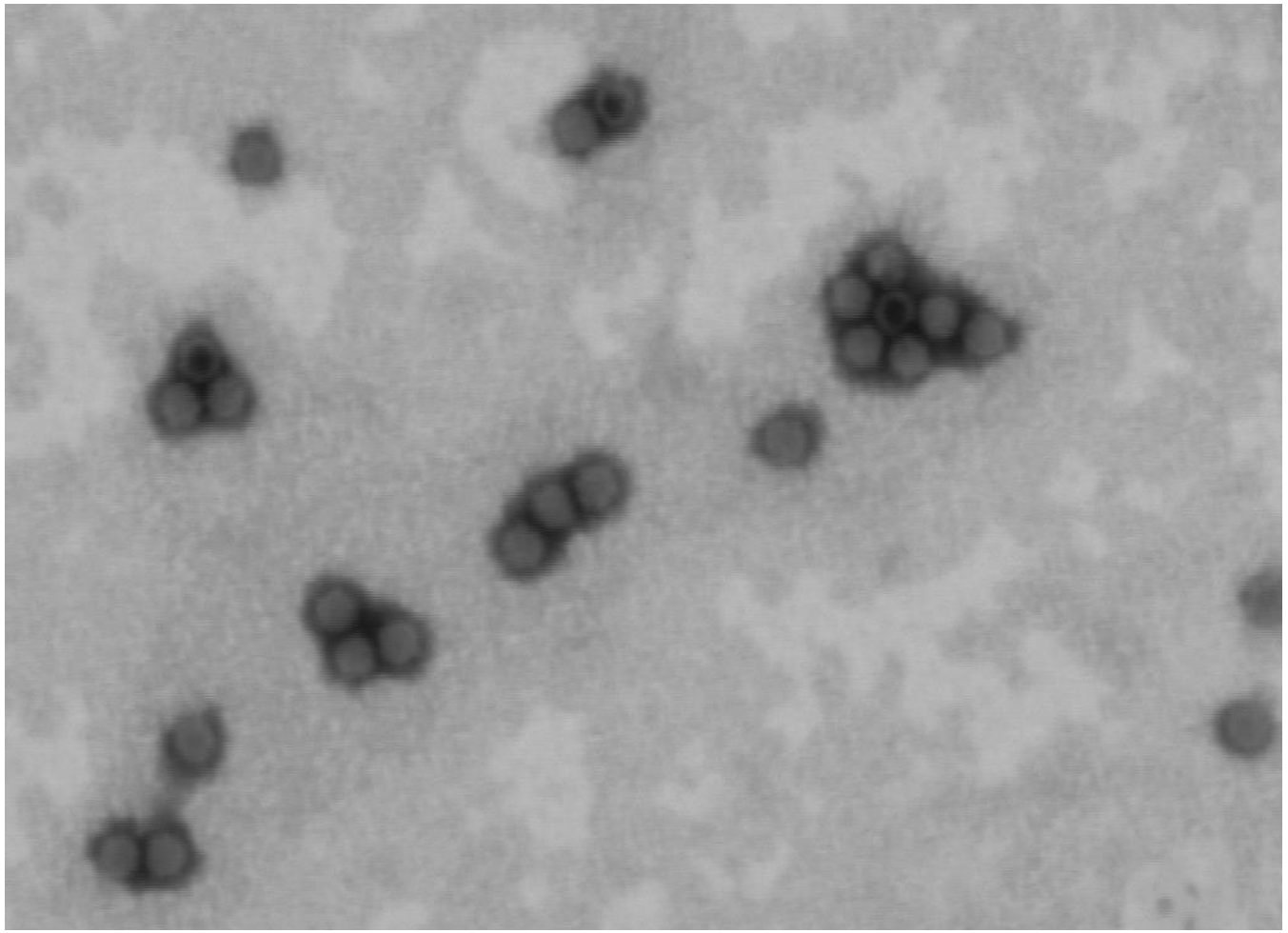

Fig. 15 nsTEM image of AAV particles (LVEM 25)

- Full-empty ratio
- Agglomeration/particle size
- Integrity

6 Conclusion

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TEM for viral safety

- **Holistic approach (Particle classification, purity etc.)**
- **Direct, fast & cost-effective analysis**
- **GMP validated**

TEM for gene therapy products

- **Minimal sample volume required (5 µl)**
- **One method for multiple critical product attributes**

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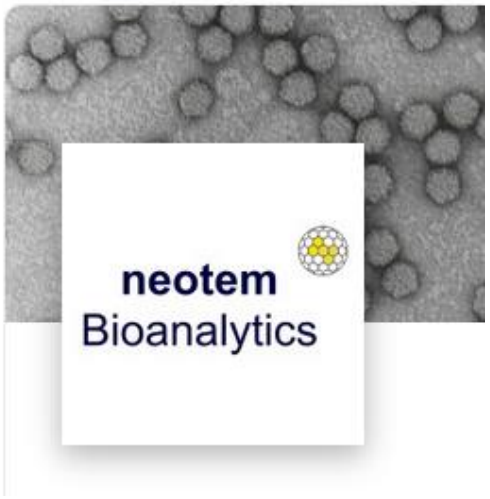
Exploring the Invisible: TEM expertise for powerful sample analysis



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Sources

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1. Escalante, Cristian & Sierra, Escalante. (2019). Fundamentals of transmission electron microscopy, the technique with the best resolution in the world.
2. Kazuyoshi Murata, Matthias Wolf, Cryo-electron microscopy for structural analysis of dynamic biological macromolecules, Biochimica et Biophysica Acta (BBA) - General Subjects, Volume 1862, Issue 2, 2018
3. https://www.gu.se/en/core-facilities/centre-for-cellular-imaging/electron-microscopy/negative-staining (Access: 02-APR-2024)