Transmission Electron Microscopy Miniature

▸▸ Purity analysis from development to commercial process

Transmission electron microscopy (TEM) can provide unique insights when characterizing viral gene delivery platforms such as those based on adeno-associated viruses (AAVs). Morphological characterization can support process development by confirming purity and status of the viral capsids.
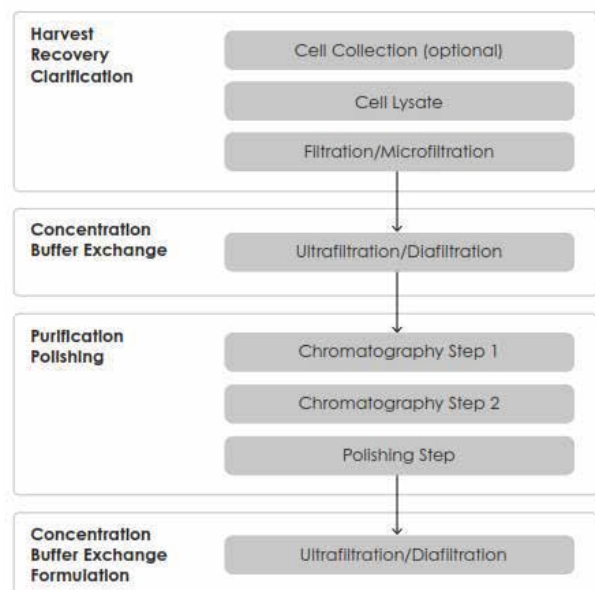

MiniTEM		VAS		
Pre-clinical	Phase I	Phase II	Phase III	Phase IV

Process development	QC and Manufacturing	Life cycle
Confirming critical quality attributes = overall AAV particle morphology and capsid status, aggregates, process and host cell related impurities	QC = full/empty analysis	Stability testing

▸▸ Example of a downstream process for AAV

• As more and more gene therapy projects move into later clinical phases, manufacturers face new challenges.When developing the commercial manufacturing process scaling up may have impact on the purity profile at different steps. Access to reliable analytical data to base process change decisions on is key to success for a smooth process development.

Impurities or morphology features in AAV

samples that can be detected by TEM:

• Broken particles

• Proteasomes

• Protein or membrane-based cell debris

• Residual DNA

• Capsid content (full/empty)

• Aggregates

• Remnants of helper virus

Harvest Recovery Clarification	Cell Collection (optional)
	Cell Lysate
	Filtration/Microfiltration

Concentration Buffer Exchange	Ultrafiltration/Diafiltration

Purification Polishing	Chromatography Step 1
	Chromatography Step 2
	Polishing Step

| Concentration Buffer Exchange Formulation	Ultrafiltration/Diafiltration

▶▶ Miniature in process development

- Experience from providing an EM service for gene therapy vectors, and of the type of features that need to be characterized frequently and in high volume, has inspired AS to develop Miniature. Miniature is a low voltage system that can be placed in any standard lab, close to your process. Miniature provides bioprocess workers with their own in-house solution that enables non-experts in electron microscopy to generate quantitative data and images in a few hours - data that can support decision making in process development.

Miniature:

- Automates imaging, particle detection and classification
- Visualizes your product and confirms critical quality attributes
- Turns visual evidence into quantitative data
- Generates instant results that allow you to adjust the process within hours

## ▶▶ Monitoring each step in the purification of AAV

Negative staining (TEM) is a powerful method for assessing process- and product-related impurities. It is a quick and easy preparation technique, supported by Miniature, in which the specimen is embedded in a layer of heavy metal salt solution. The use of heavy metal stain significantly increases the contrast in the images.
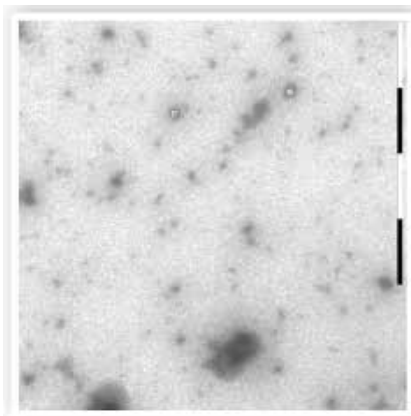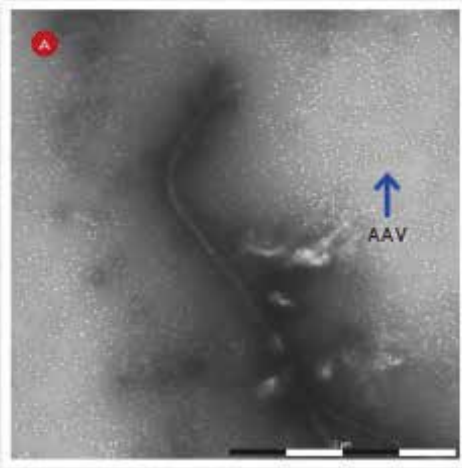
Sample after 1st
purification step
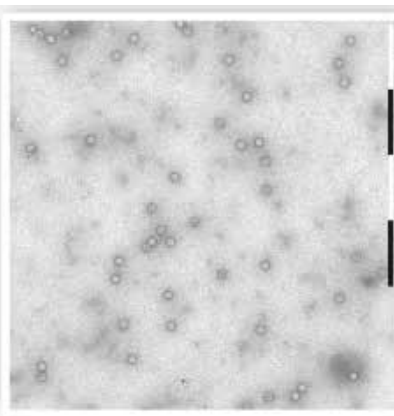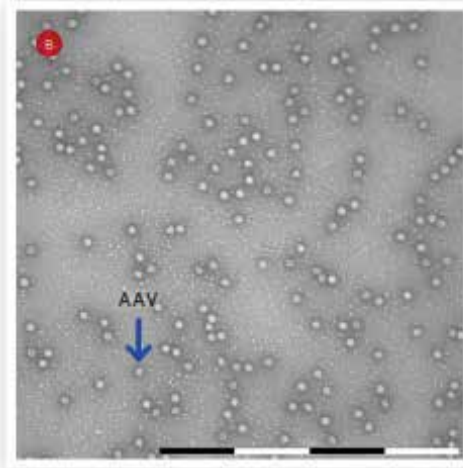
Sample after 2nd
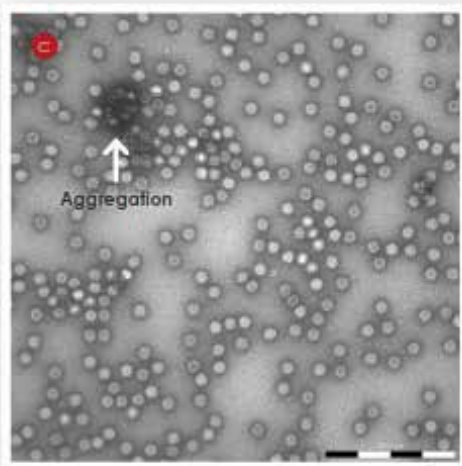purification step
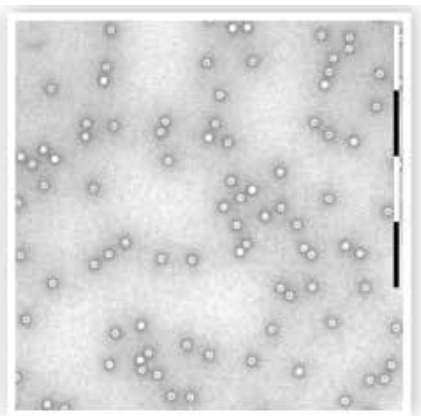
Sample after final
purification step

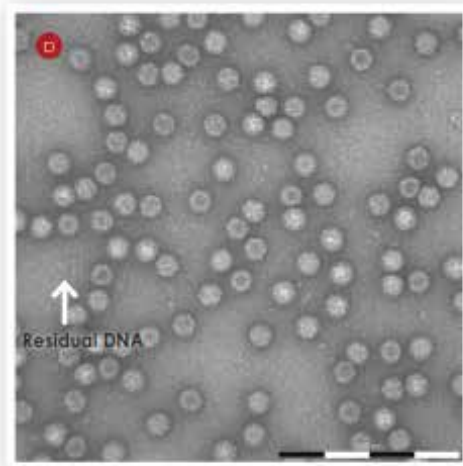## ‣‣ The types of contaminants that may appear

(A) Large size impurities, 4 μm

(B) Small size impurities, 2–15 nm.

(C) Aggregates



(D) Residual DNA impurities, 2 nm thickness

Undesired sample characteristics to consider when optimizing a process
• Aggregation
• Failure to remove host-cell debris or residual DNA
• Loss of particle morphology or integrity

All these types of contaminants can easily be identified and quantified by using the automated image analysis software tools in Miniature.

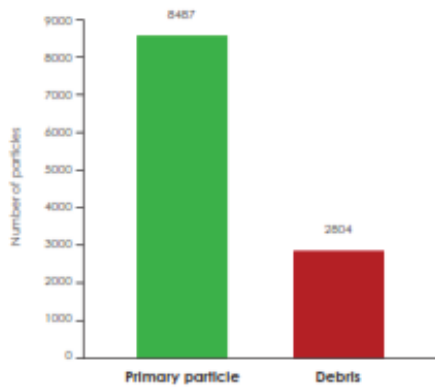## ▸▸ Automated analysis of purity and level of debris

Miniature automatically images, detects and classifies particles and debris. Since automation enables detection and analysis of a far larger number of particles than is feasible with manual operation, the results are more consistent, statistically significant and reproducible.
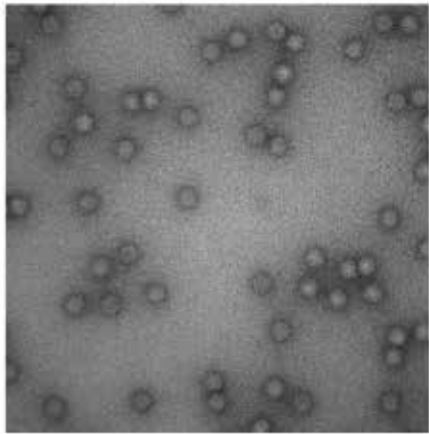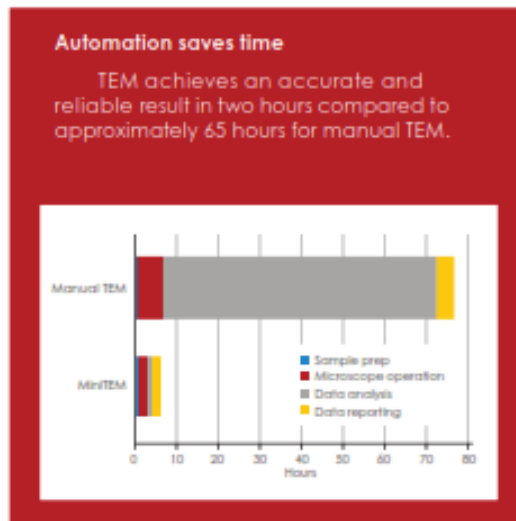
Undetected particles
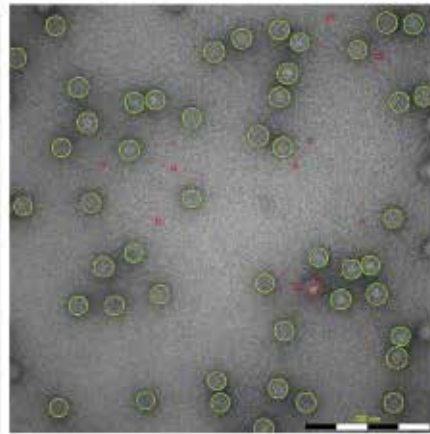
Detected primary particles and debris

Particles detected, classified and quantified using Miniature.

Automation saves time

TEM achieves an accurate and reliable result in two hours compared to approximately 65 hours for manual TEM.

Manual imaging on conventional EM was not performed in its entirety, for practical reasons. In order to compare the time required to obtain data of similar accuracy using manual handling, the time for data analysis of 20 images was extrapolated to estimate the time that would be needed for 504 images.

▸▸ Proteasomes could be hiding in your AAV sample

Comparing Miniature analysis with DLS size distribution analysis on the same sample demonstrates that the quanititative data based on image analysis by Miniature can reveal the presence of proteasomes where DLS gives only one broad peak.
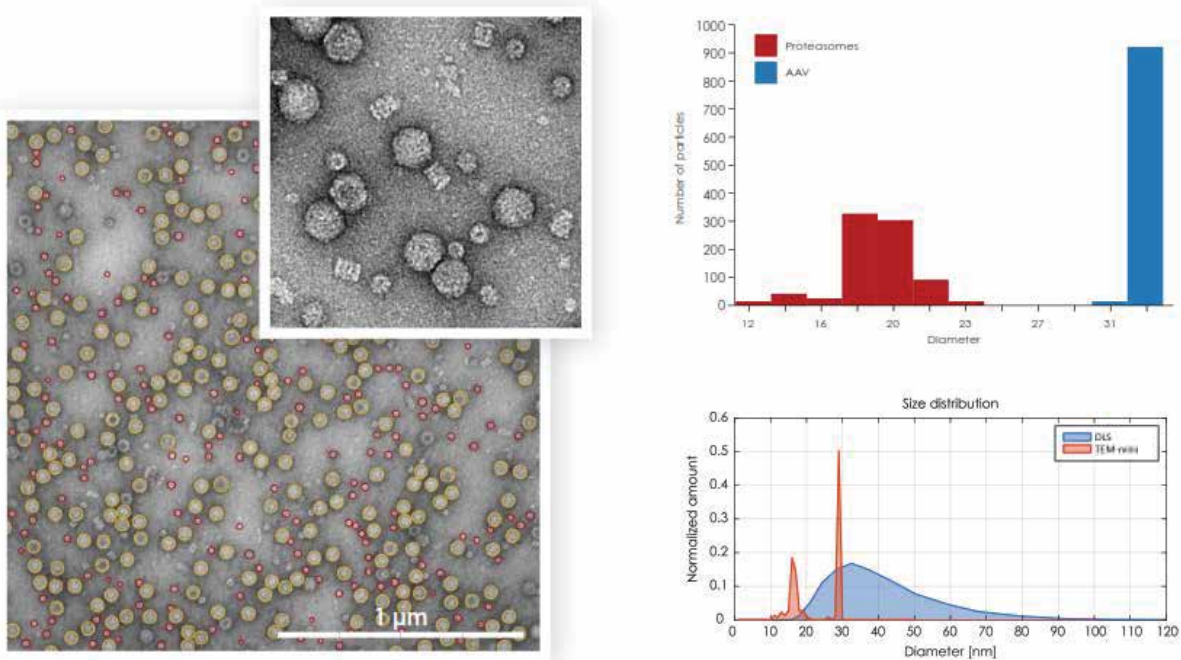
▸▸ Size distribution analysis to reveal aggregates and debris

Particles present in the sample that deviate from the expected size and shape can be the undesired outcome of an un-optimized process step. In this example a large aggregate as well as smaller size debris are detected and automatically measured.
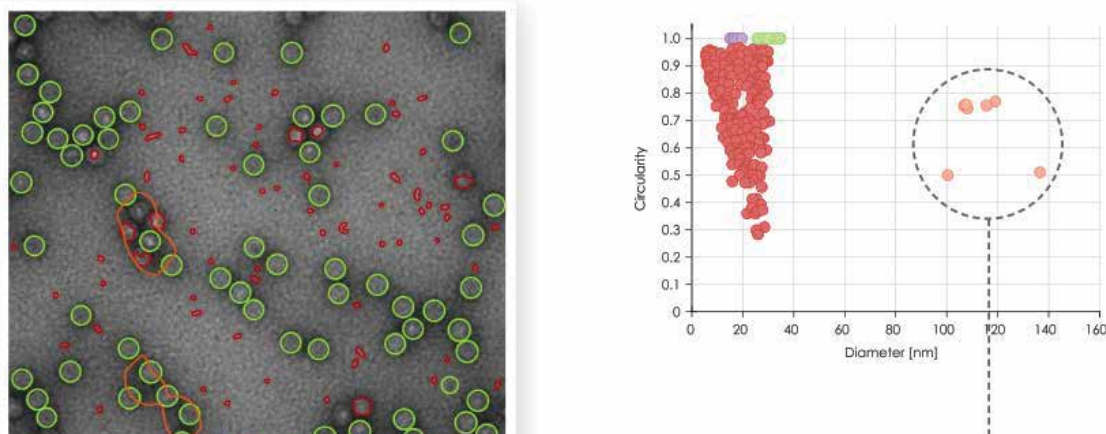

Outliers represent potential aggregates. By clicking on the outlier Miniature takes you to the image and you can determine whether it's a true aggregate or not.

▸▸ AAV capsid integrity variations observed with Miniature

Negative-stained samples of AAV particles display variations in staining patterns that provide a clear indication of capsid integrity status. Intact AAV particles appear as lacking internal staining as opposed to broken AAV particles that exhibit internal staining because of presumed disrupted virus capsid structure. Particle doublets and triplets that can occur under certain conditions can also be detected.
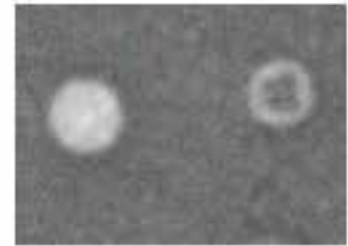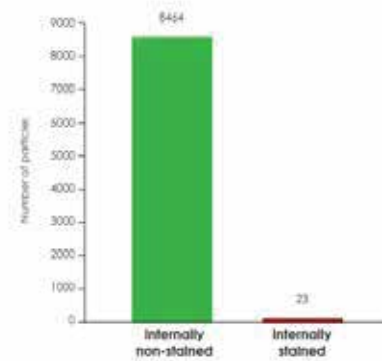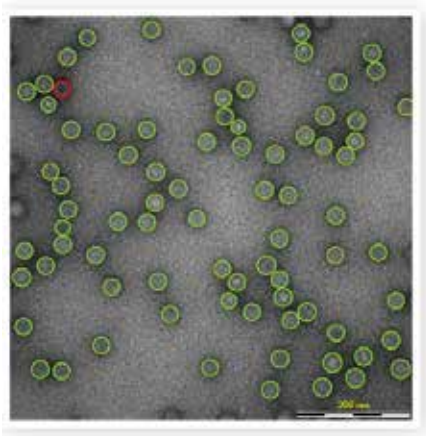
▸▸ Using Miniature to compare the capsid status in two different samples
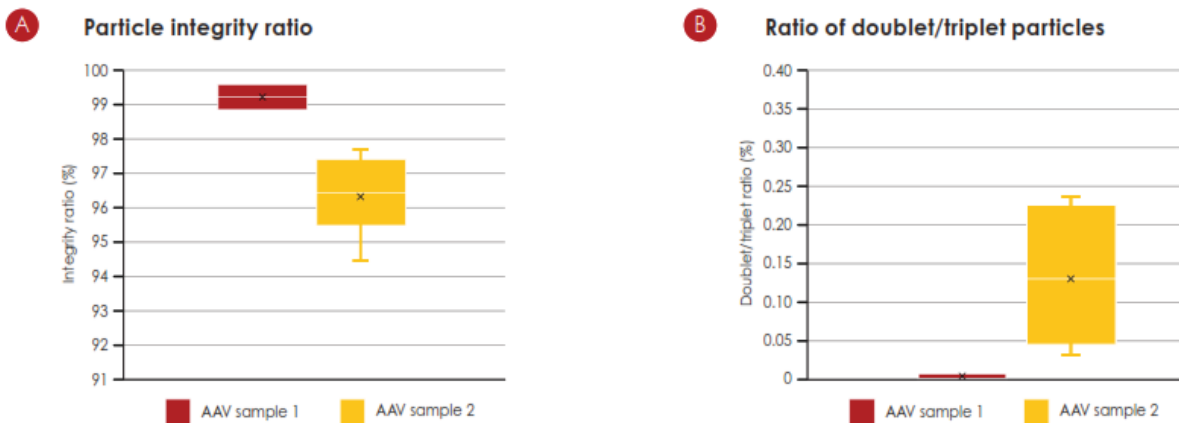
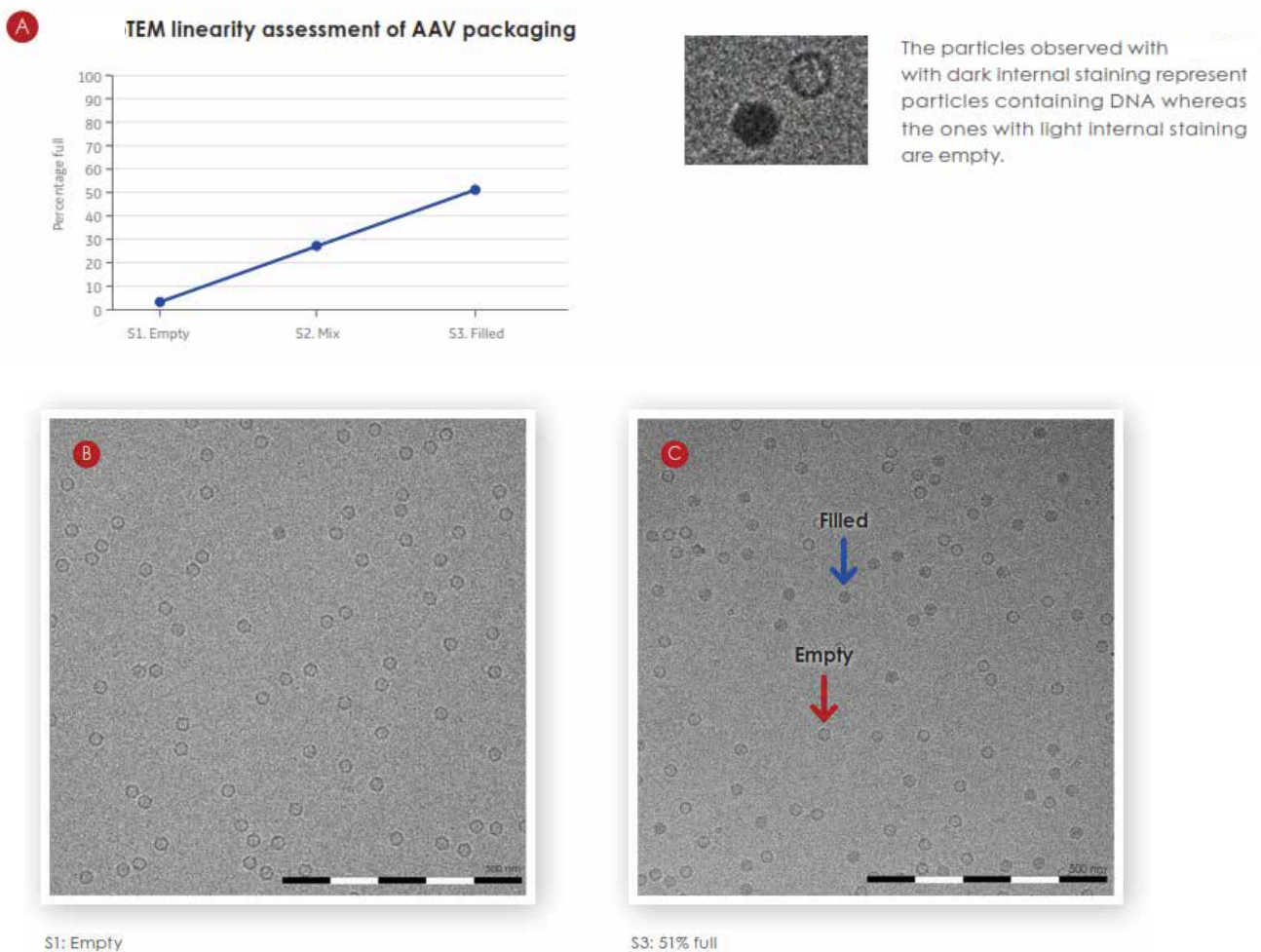In this Miniature study, the integrity of two different samples of purified AAV particles was automatically quantified and compared.

The study showed that AAV Sample 1 had a larger portion of intact particles (A) compared with Sample 2 (99% intact particles compared with 97% in Sample 2. (B) No doublets or triplets were detected in Sample 1, whereas 42 doublets or triplets (0.125%) were detected in Sample 2.

Linearity proves accuracy of TEM full/empty analysis

TEM is a method by which the biological specimens are vitrified by rapid freezing in liquid ethane, embedding the molecules in a layer of amorphous ice. TEM-method does not involve using stains or other chemicals, so therefore samples prepared in this way preserve their native structure. The particles observed with TEM-method with internal dark staining represent particles containing DNA. TEM-method in combination with AS Analyzer Software (AS) has been validated for quantification of percentage full AAV analysis. In this example three different samples are analyzed: empty (S1), filled (S3) and a third sample that is a mix 1:1 of sample S1 and S3 (S2). The TEM-method shows the expected linear relationship between particle content and staining.

The particles observed with with dark internal staining represent particles containing DNA whereas the ones with light internal staining are empty.


S1: Empty

S3: 51% full

The quantitiative image analysis of 3 imaged samples defined as empty (S1), 51% filled (S3) and a mix of S1 and S3 (S2) performed with AS is shown in the graph in figure (A). The imaging results are shown in figure (B) and (C) below.

▸▸ Validated AAV full/empty analysis service

Virus particle packaging analysis with TEM-method in combination with AS is recommended for batch release testing and QC of AAV-based gene therapy products. The automation that AS provides allows AS EM services to deliver high quality images and timely and cost-effective accurate quantitative data.

Scatter plot showing 2 distinct populations of particles; full in red and empty in blue. Particles that don't cleary belong to any of these groups and which appear between the red and blue clusters are identified as uncertain.
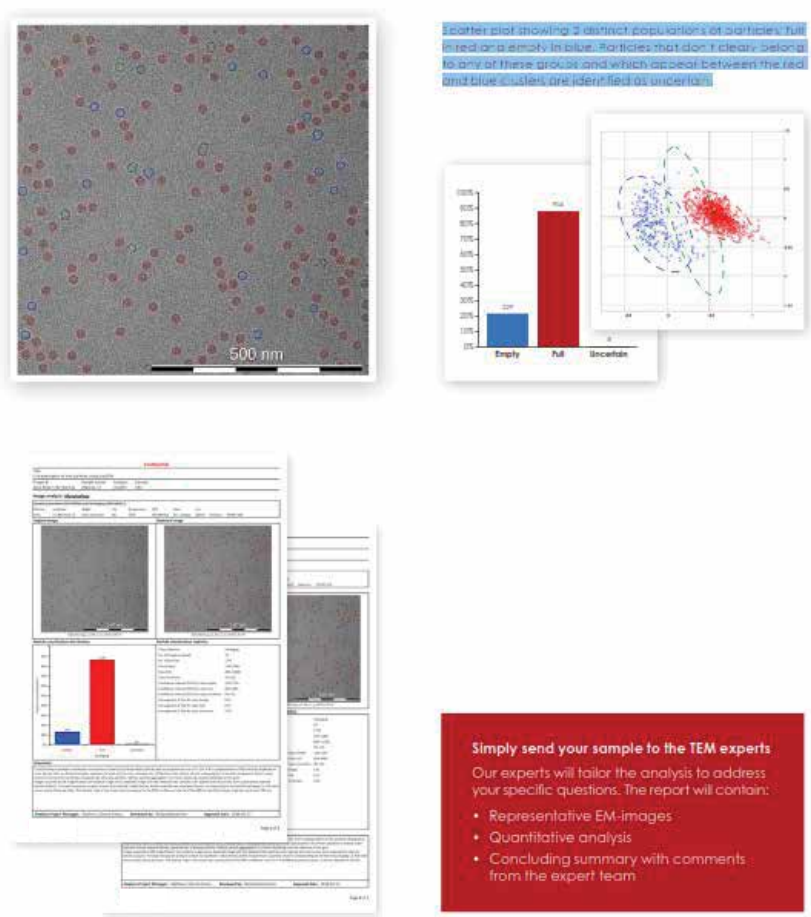
▸▸ Analyzer software

analyze transmission electron microscopy (TEM) images of nanosized particles.
enables reproducible and semi-automated particle detection and classification. For QC testing of capsid percentage full/empty of AAV samples AS offers services using TEM-method and image analysis that can be validated according to GMP.

Detect	Explore	Report	Complicance with GMP
The software works by algorithmically identifying particle characteristics based on selected criteria, such as the size or shape.	Each detected particle is stored together with the meta data. You can use the collected data to qualify particles into separate classes or groups, such as filled or empty.	After the analysis generates a report of the results with particle data presented in histograms, with statistics, together with representative microscopy images.	The software is 21 CFR part 11 compliant and has access control, electronic signature, full traceability and audit log. suitable for regulatory controlled environments that work under GMP.

Full traceablility

- Database architecture for safe archiving
- Instant import of images from live microscope session
- Pre-defined roles for access and right to process data and sign off
- Electronic sign off

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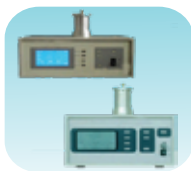| UV/VIS Spectro 2080+ Double Beam | Infra FTIR | Optima Gas Chromatograph 3007 | Optima Gas Chromatograph 2979 Plus | Flash Chromatograph | Atomic Absorption Spectrophotometer | Liquid Partical Counter |

| Optical Emission Spectrophotometer | DSC/TGA | Semi Auto Bio Chemistry Analyzer | HEMA 2062 Hematology Analyzer | Micro Plate Reader/Washer | URINOVA 2800 Urine Analyzer | Total Organic Carbon 3800 |

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