Vironova

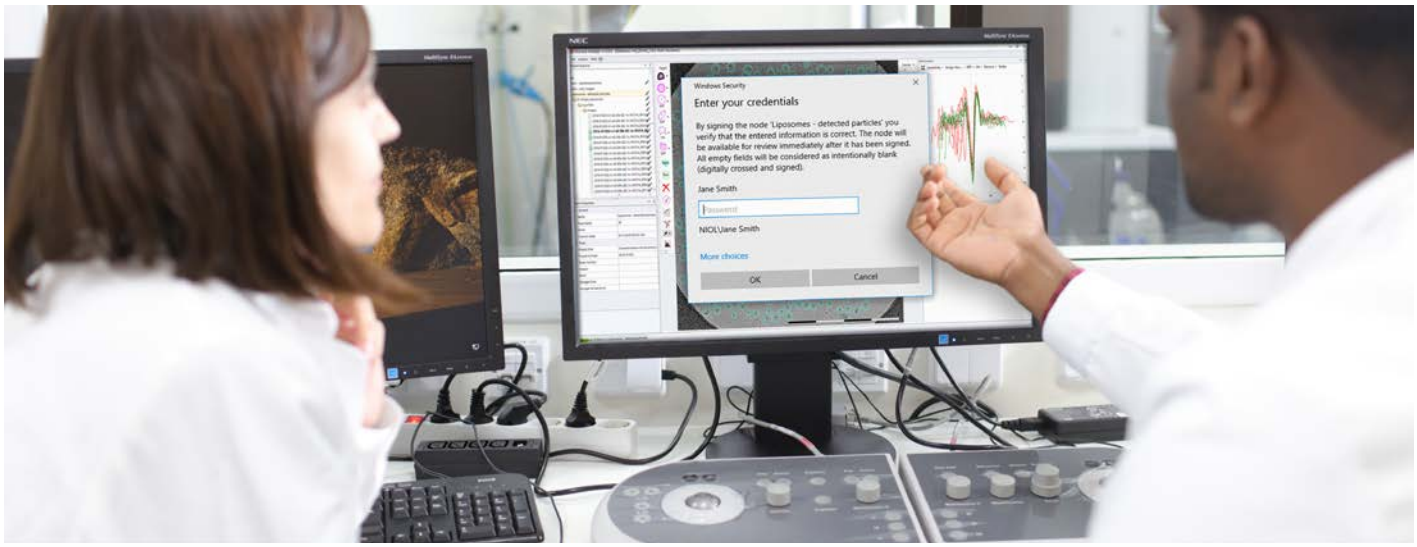
VAS

Vironova Analyzer Software

FDA 21 CFR Part 11 compliant software for TEM based image analysis of nanoparticles

Vironova Analyzer Software (VAS) is a tool for computer-assisted analysis of transmission electron microscopy (TEM) images in a GMP compliant environment.

VAS is suitable in regulated environments within the Life science industry where establishment of procedures and controls that ensure integrity, confidentiality, authenticity and reliability in their electronic records according FDA 21 CFR part 11 regulations are required.



How to use VAS

Analysis of objects in the images can be performed using a combination of automatic analysis tools based on mathematical algorithms, and manually operated tools that aid a domain expert in assessing the images.

The operator can use VAS to analyse batches of images, and to gather quantitative measures of objects in the images as well as generating graphs and statistics based on those measurements. The result of the analysis is stored in a database and can be gathered into an electronically signed customized analysis report. The analysis report can be exported to digitally signed files.

After completed transfer of images from microscope to VAS, every processing and annotation operation is recorded in a database for persistent storage. Original data and each subsequent version of data records are saved so that there is always a way to trace earlier versions in a secure audit trail. Members of certain user roles can review data, electronically sign the analysis result and gather data into an analysis report.

System access

The database administrator can assign users and user roles on a user-by-user basis. VAS provides the following user roles: Analyst, Coordinator, Reviewer and Superuser.

All settings in VAS regarding electronic signatures, user roles, authentication, re-authentication, system user uniqueness and passwords are done using integrated Windows authentication and authorization functionality.

Authority checks

All users of VAS will be authorized by their Windows domain credentials before they can use the software. VAS will not start unless the Windows user logged onto the computer is registered as a valid user for the selected database and role.

Continuous import of images and retention of data

It is possible to define if a specified database in VAS should only accept continuous import of images from live microscope sessions. This ensures that the images are not processed or modified before being persisted in the database.

Data, i.e. images and meta data transferred from a transmission electron microscopy (TEM), is saved in VAS prior to any changes by the user. Thereafter, each version of the data is also stored as changes are made.

Audit trail

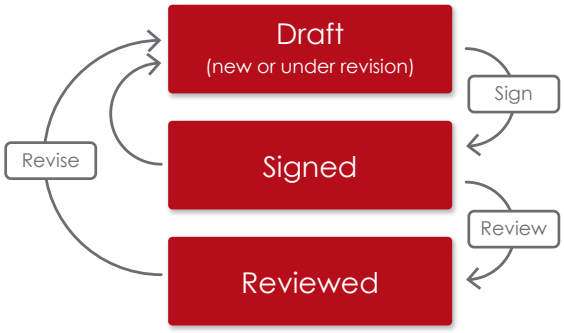
Data entered into VAS by a user and data generated as a consequence of running an automatic or semi-automatic function in VAS are saved immediately to the database. The data in the database cannot be changed or deleted.

Every user action in the database is compiled into an audit trail table which includes VAS version, user name, user role, date and time and specific details of the action. The audit trail can be viewed in VAS or be exported to Microsoft Excel or other similar program.

Electronic signatures

Analysis reports must be electronically signed by authorized users.

At a given point in time, data resides in one of three different states: Draft, Signed or Reviewed. Access to the different transitions are given to a user based on their user role and VAS requires the user to electronically sign all transitions between the different states.



Signature information includes the name of the signer, the date and time of signature execution and the meaning of the signature. The electronic signature requires the user to provide the same credentials as when logging into the computer running VAS. All electronic signatures are persisted in the database and linked to their respective electronic records.

Data export

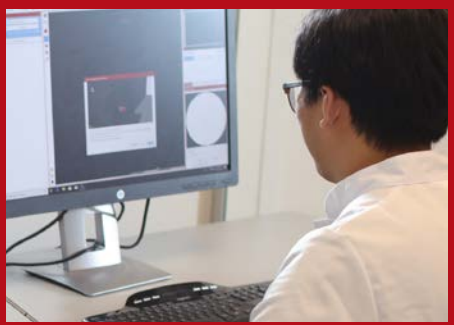
Images, analysis results and reports can be exported to a relevant file format. The analysis report can be exported to digitally signed files.

Only accumulated statistics is visible in the analysis report, but the detailed underlying data is possible to export to a csv file and open in Microsoft Excel.

Data in the audit log can be sorted, filtered and exported to Microsoft Excel.

VAS helps your organization comply with 21 CFR Part 11

- Access to the system is controlled and different user roles are supported
- Continuously import of acquired images
- Data cannot be changed or deleted
- Audit trail is maintained of all operations carried out by the users
- Electronic signatures can be applied
- Digitally signed analysis reports can be generated



Automation enabled by machine learning

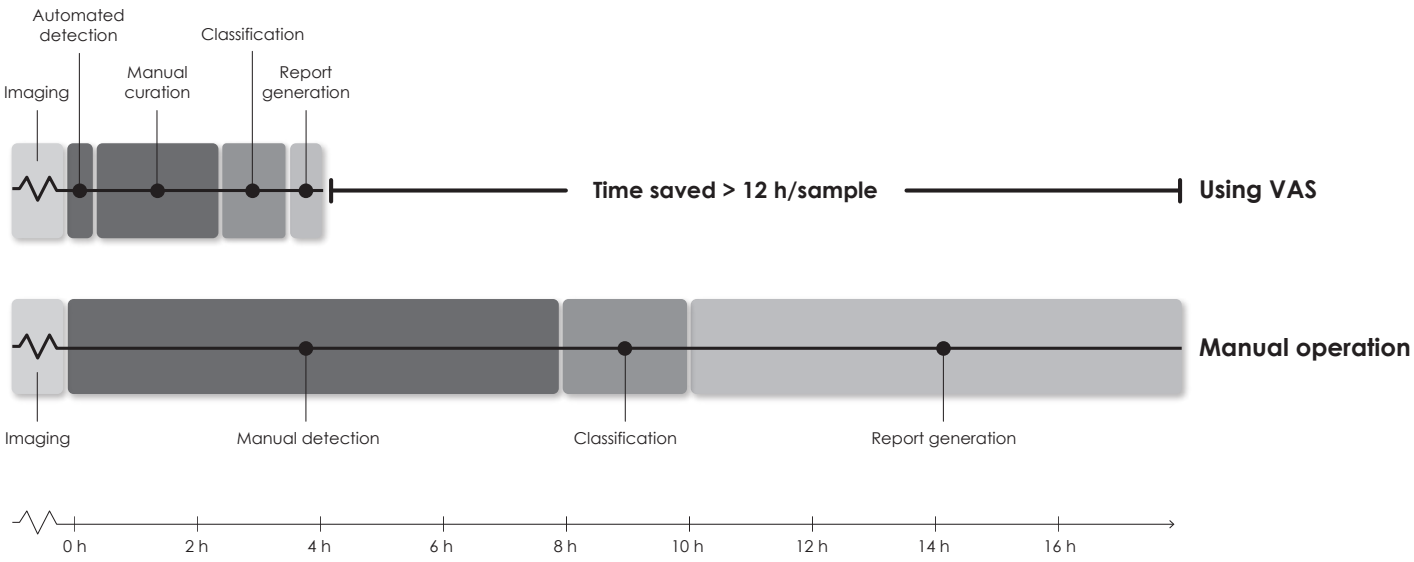
VAS comes with machine learning capabilities making it possible to set up methods to automatically classify particles. The machine learning method used is called support vector machines (SVM) and is a supervised learning method. This means that the classifier must be trained on a set of manually classified particles before it can be applied to new, unclassified particles.

The trained classifier is proven for the ability to discriminate between filled and empty particles. To do this discrimination it uses a predefined combination of features including shape, size and intensity profile.

The classifier can be applied to all particles in an image or to individual particles separately in a similar way as the manual classification tool.

Time saved with VAS

Significant time can be saved with VAS. In this example it is shown the time required for the analysis of liposome lamellarity in 20 images of a sample with average quality.



Compliance with GMP

Detect

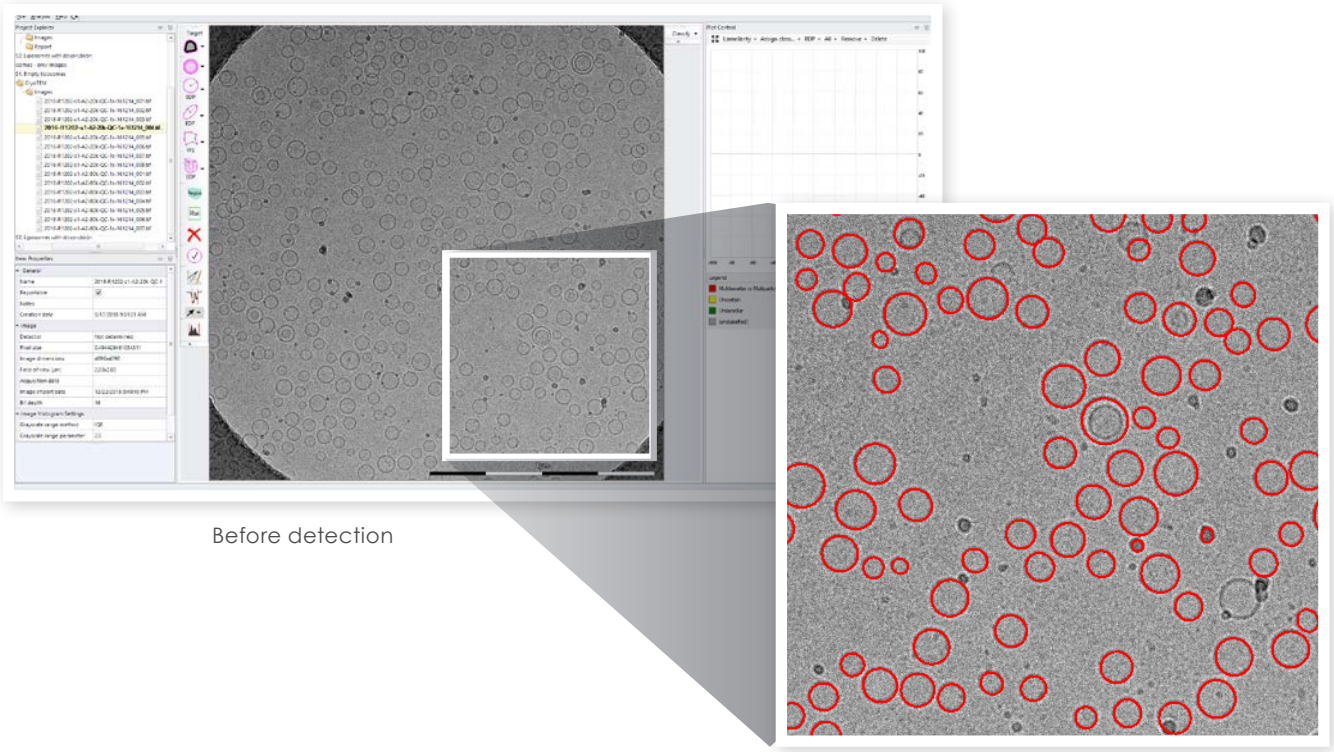
Explore

Report

Detect nanoparticles in TEM images

Images acquired with TEM of a sample of nanoparticles can be analyzed with VAS. The tools allow you to manually or automatically detect particles in the image based on pre-selected criteria such as

- size
- shape
- intensity profiles

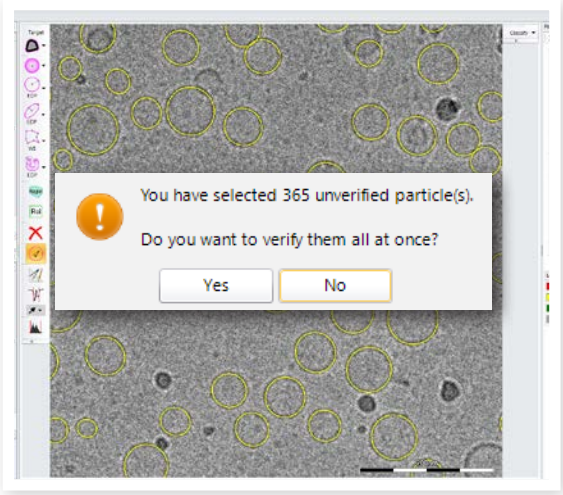


Before detection

After detection

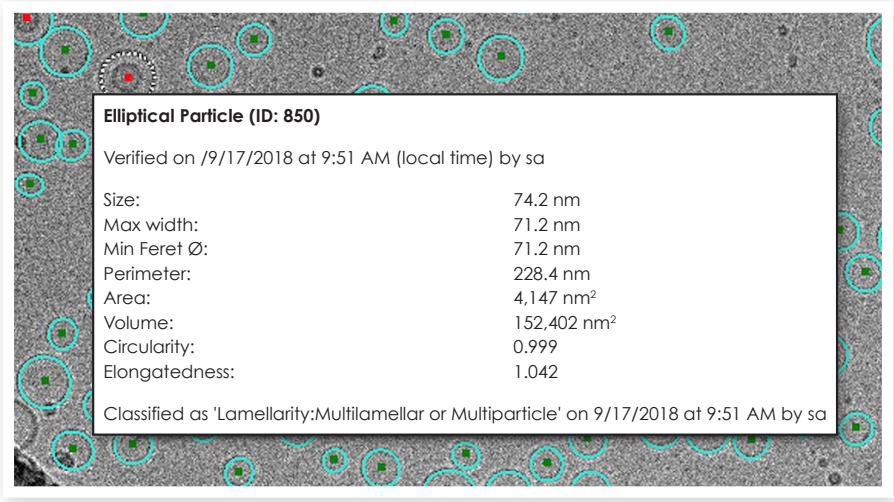
Tools for curation and verification of particle detection

After the detection is completed, you can easily go back and verify that the particles have been correctly identified. In case of false positives you can simply delete or in case of particles that remain undetected you can also simply add them. You can choose to verify particles one by one or in batch.



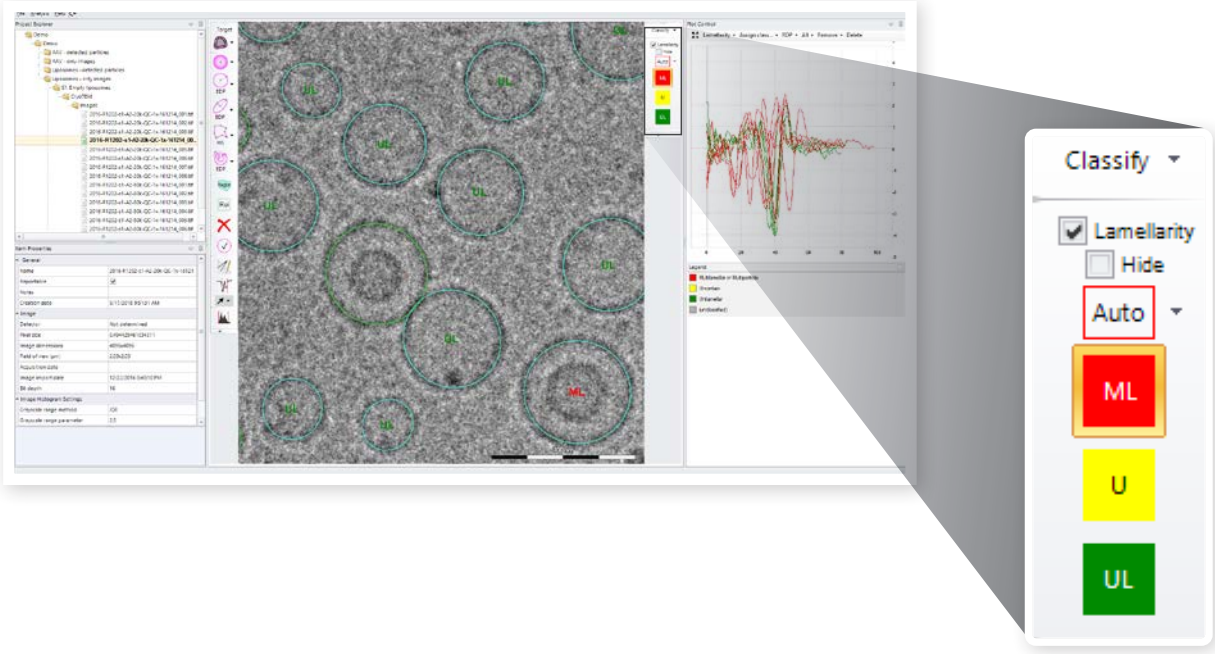
Explore image data to classify your particles

The features that have been selected for the particle measurement and detection are safely stored in a structured way in the VAS database. Data for each individual particle that has been detected can easily be found and tracked. The data can then be used to further explore the feature space and to classify particles.



The classification uses the PCA tool to recognize in what dimenstion the particle populations are distinct. Some examples of analyses that can be performed by this classification include:

- Size distribution
- Circular or elongated liposomes
- Mono- or multi-lamellarity of liposomes
- Filled or empty analysis of AAV or Liposomes

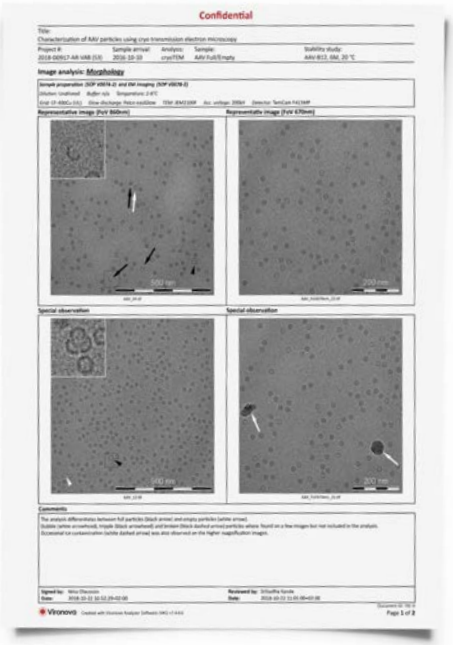


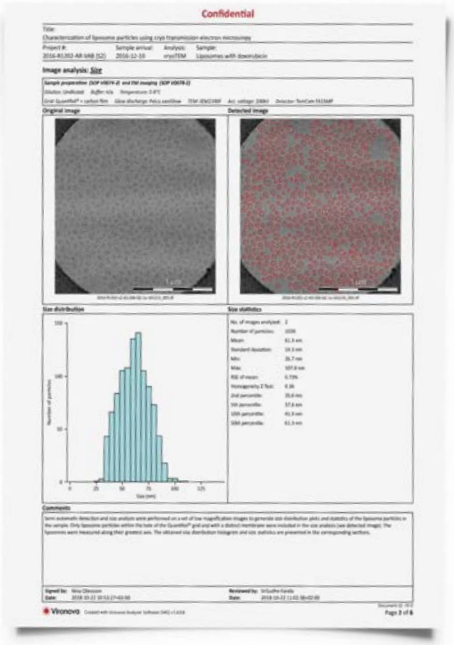
Report

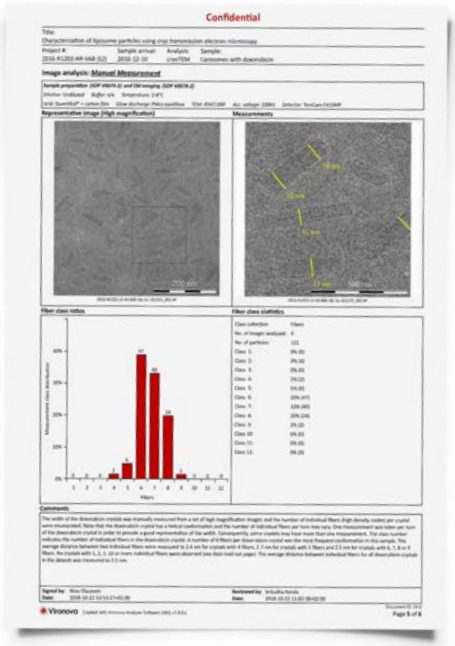
Report generation with electronic signature

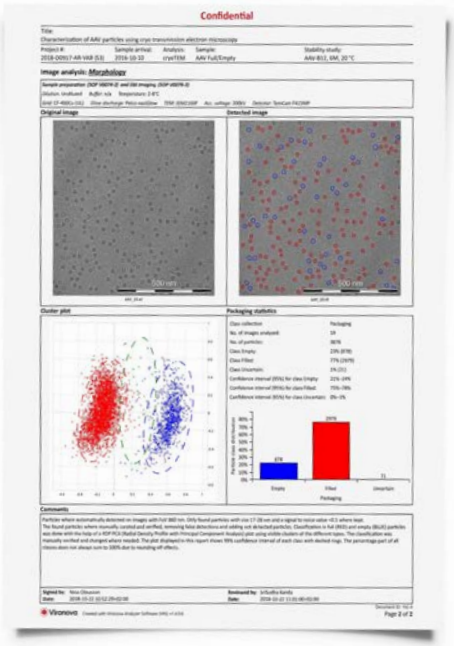
A report can easily be generated that presents the analysis result

- The particle data and class distribution as histograms
- Representative TEM images
- Graphical presentation of the results
- Statistical measures such as mean and variance
- Electronically signed off in compliance with 21 CFR part 11





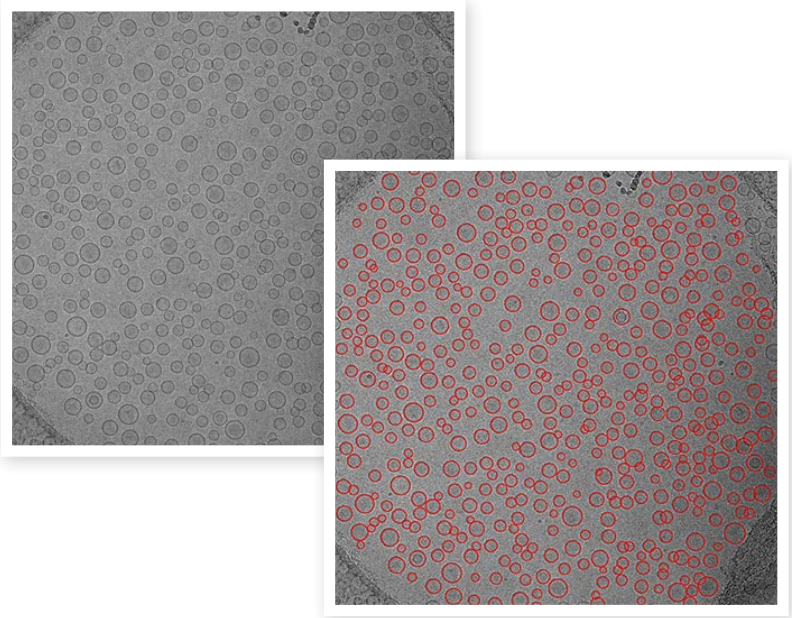




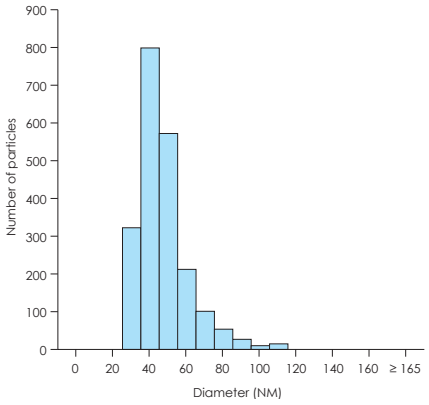
Application example liposomes

CryoTEM combined with image analysis using VAS for objective analysis of liposomal drug products

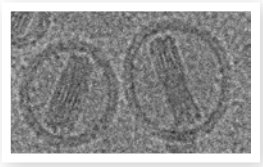
Size distribution

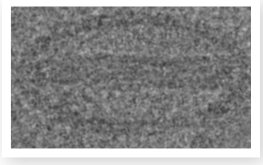


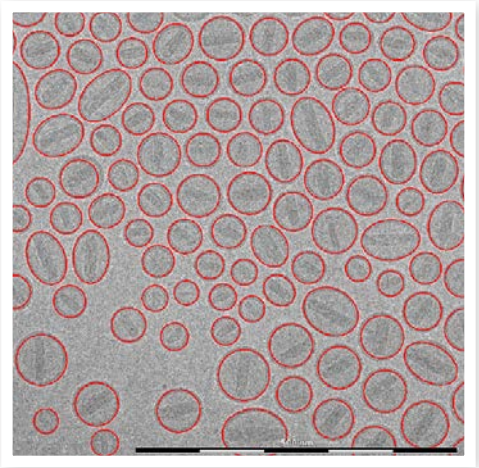
No. of images analyzed:	3
Number of particles:	2140
Mean:	47.66 nm
Standard devilation:	15.49 nm
Min:	28.75 nm
Max:	201.10 nm



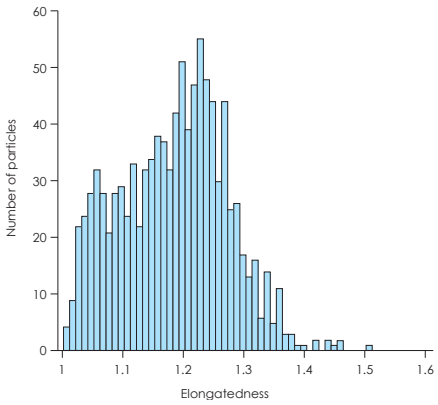
Elongation distribution



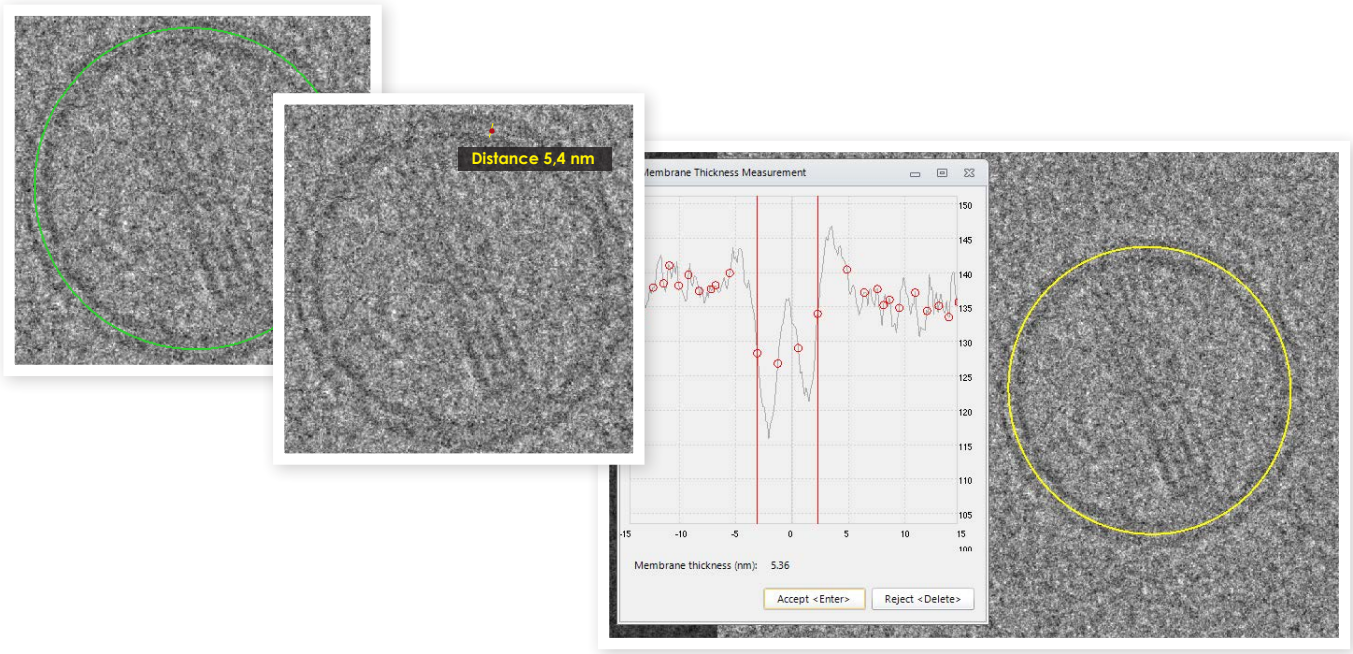




Number of images analyzed:	2
Number of particles:	1026
Mean:	1.19
Standard deviation:	0.09
Min:	1.01
Max:	1.51
RSE of men:	0.23%
Homogeneity Z-Test:	1.40
2th percentile:	1.03
5th percentile:	1.04
10th percentile:	1.06
50th percentile:	1.19
90th percentile:	1.29
Span:	0.20

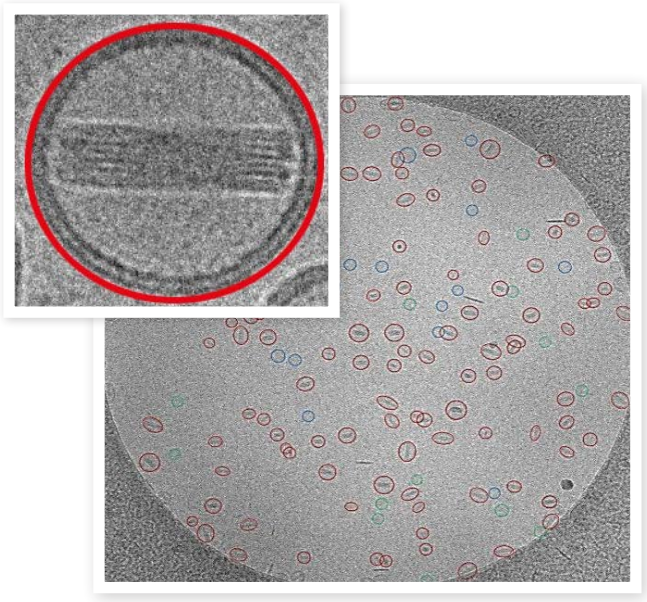


Semi-automatic membrane thickness determination

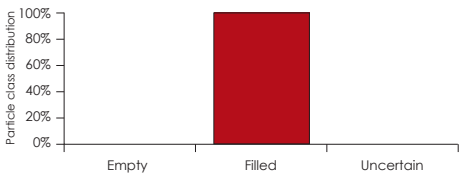


Proportion of filled particles

Particle classification

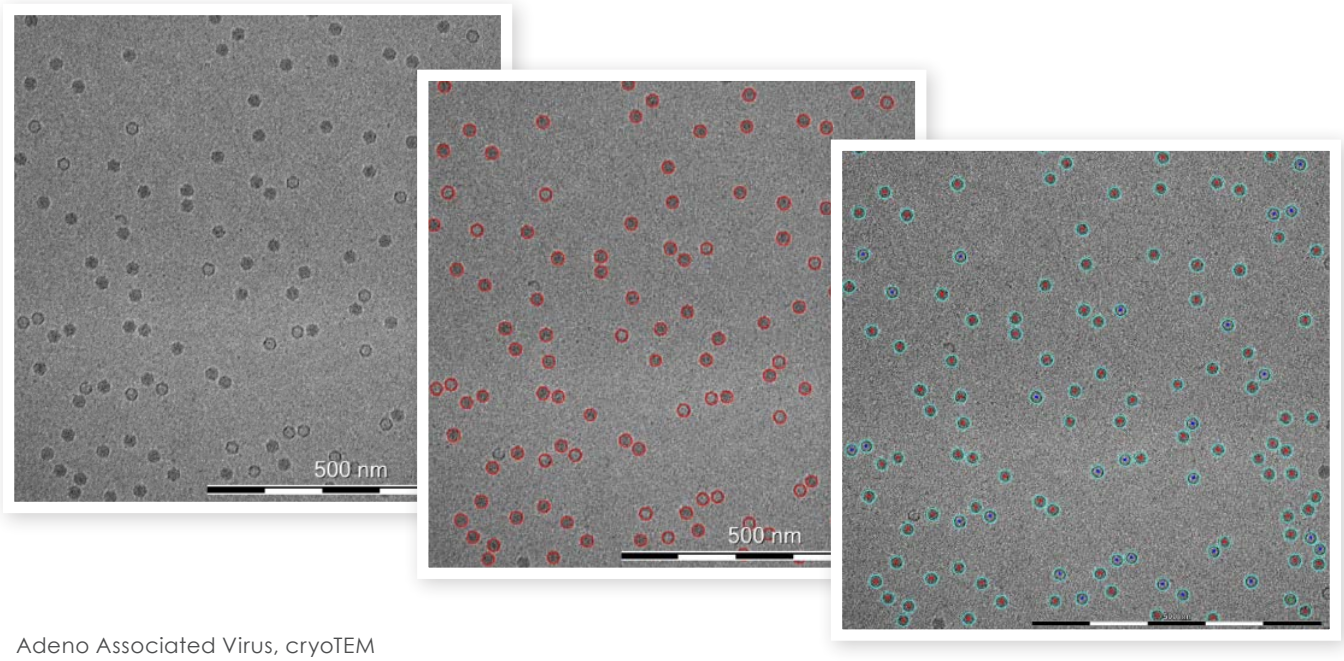


Number of particles:	1026
Class empty:	0% (4)
Class filled:	99% (1015)
Class Uncertain:	1% (7)
Homogeneity Z-Test for class Empty:	0.00
Homogeneity Z-Test for class Filled:	0.91
Homogeneity Z-Test for class Uncertain:	1.14

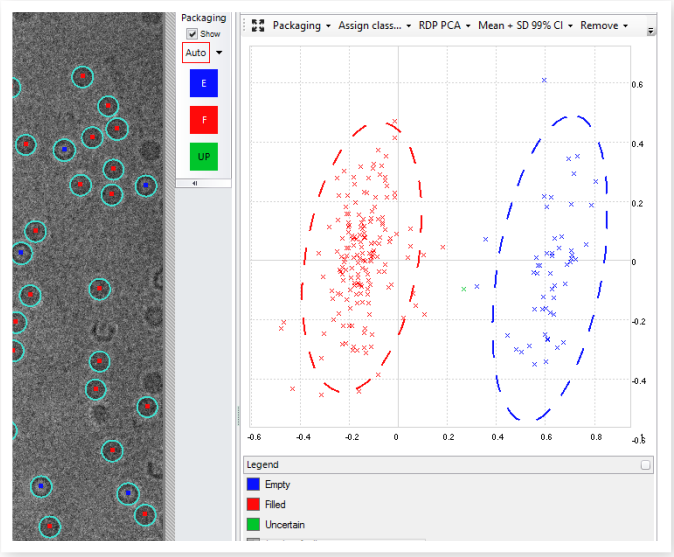


Application example full/empty analysis capsid AAV

Virus particle packaging analysis with cryoTEM in combination with VAS is recommended for batch release testing and QC of AAV-based gene therapy products.

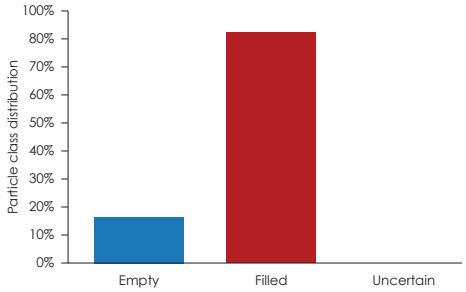


Adeno Associated Virus, cryoTEM



Proportion of filled particles

Class collection	Packaging
No. of images analyzed:	13
No. of particles:	1502
Class Empty:	21% (319)
Class Filled:	78% (1174)
Class Uncertain:	1% (9)



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