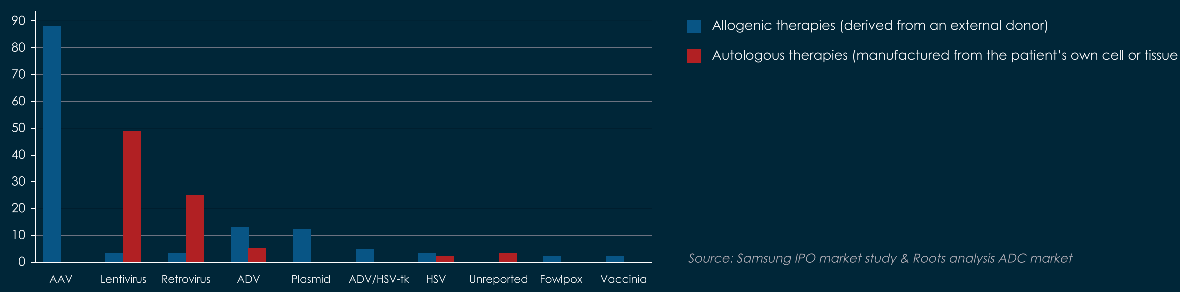
USING NEURAL NETWORK TO ANALYZE LENTIVIRUS

01.

Lenti is the 2nd most common vector used in cell and gene therapies

Vectors used in active FDA interventional clinical trials (cell & gene therapies)



Source: Samsung IPO market study & Roots analysis ADC market

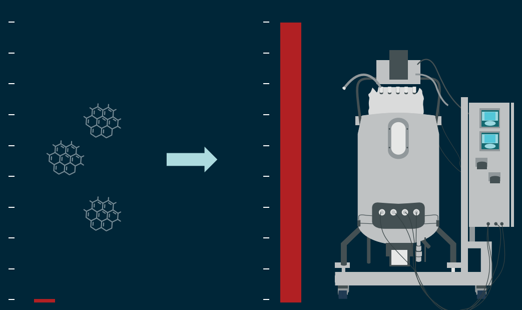
A lenti sample with high titer and purity

Vanessa Carvalho & Gustav Kylberg

Primary particle

Debris

Up-scaling Lenti virus production is a challenge



LAB SCALE
cell culture

PRODUCTION SCALE
cell culture

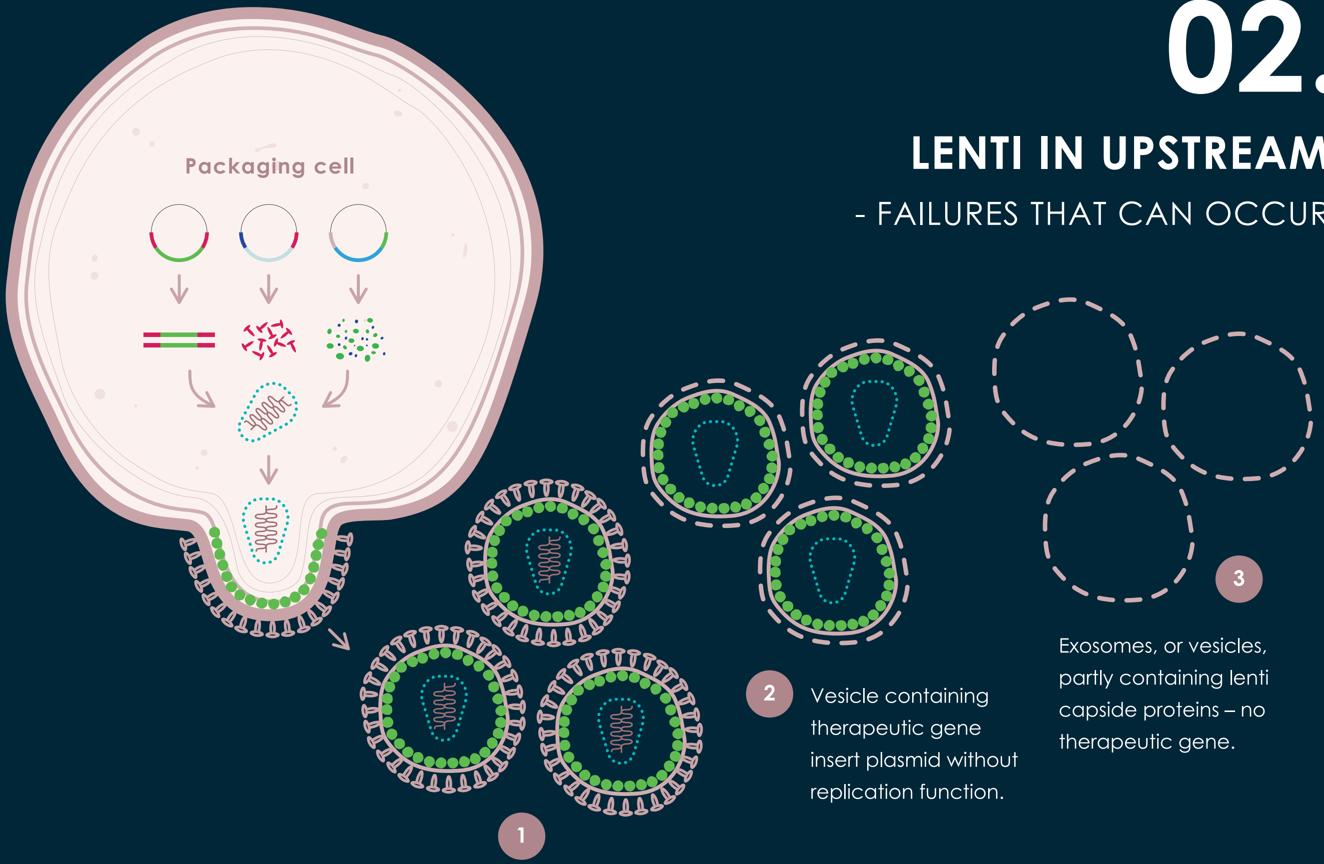
Although Lentiviruses are routinely produced at a small to medium scale in laboratories, up-scaling these methods has proven challenging.

Difficulties arise surrounding the development of stable packaging cell lines and the need for efficient purification processes that preserve vector infectivity.

02.

LENTI IN UPSTREAM

- FAILURES THAT CAN OCCUR

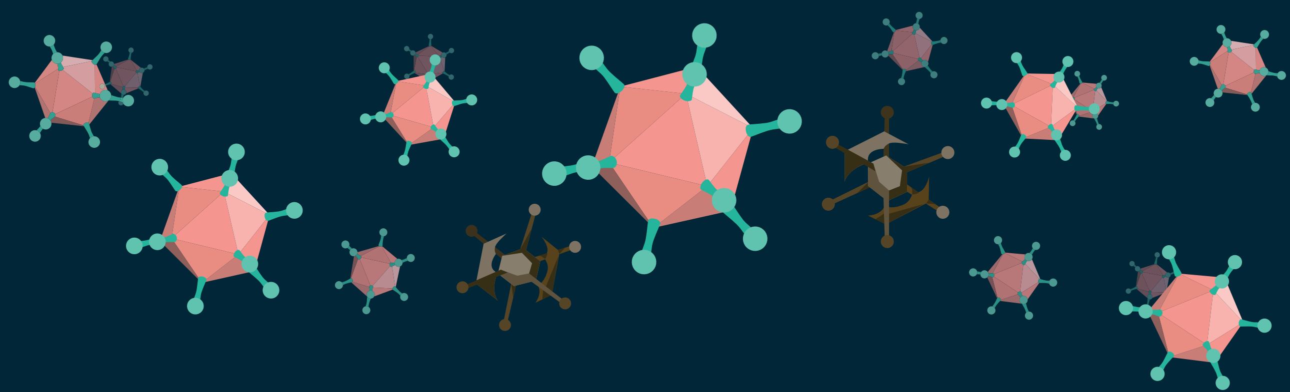


1 Lentivirus, with replication function and containing therapeutic gene insert.

2 Vesicle containing therapeutic gene insert plasmid without replication function.

3 Exosomes, or vesicles, partly containing lenti capside proteins – no therapeutic gene.

Failures can also occur in downstream



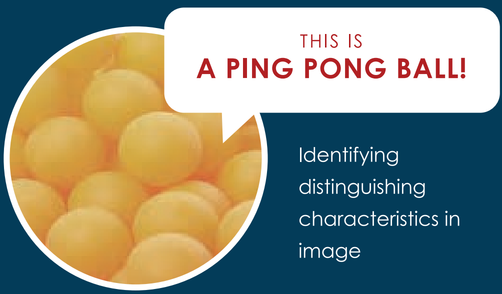
The integrity or morphology of the virus can be affected by unoptimized process conditions – accurate analysis is needed to correlate process impact on critical quality attributes.

HOW AI LEARNS TO DISTINGUISH BETWEEN A PING PONG BALL AND AN ORANGE WITH CONVOLUTIONAL NEURAL NETWORKS

03.

Convolutional neural networks (CNNs) are a form of artificial intelligence (AI) inspired by biological processes in the animal visual cortex (basically the same way that children learn to distinguish between things).

CNN uses classified data sets as "trainers"

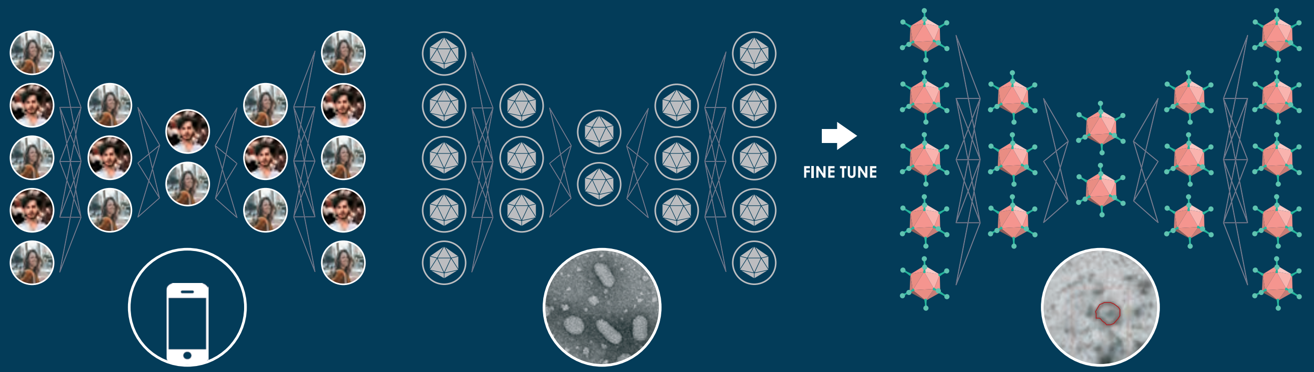


Identifying distinguishing characteristics in image

Identifying distinguishing characteristics in image

Once trained on one data set, CNNs can be trained to classify other images using smaller data sets in order to expand their applicability ("this is a box of red billiard balls", "this is a box of apples").

Vironova particle characterization using AI



Like the AI in your smartphone learns to do face recognition better and better for every picture you take, The MiniTEM learns to do virus sample analysis better by training. It starts from a good level thanks to the large image database of viral samples of Vironova.

Today only a little bit of interactive training and fine tuning is required to work on a specific application.

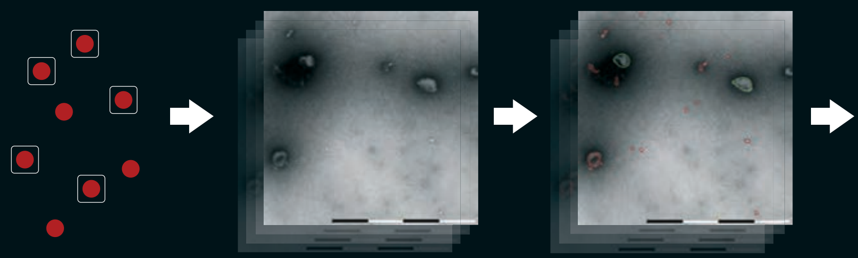
A LENTI ANALYSIS CASE:

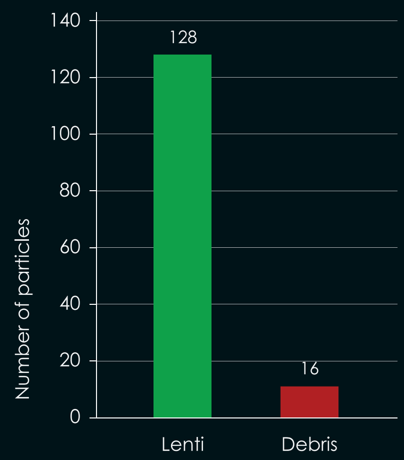
Objective: to evaluate a filter step in the downstream process.

Clarified sample → Chromatography step → Filter step

Imaging and analysis with MiniTEM™

Automated imaging with MiniTEM combined with AI constitute a powerful tool for characterizing complex samples such as Lentivirus.





Manually select areas of interest on the grid

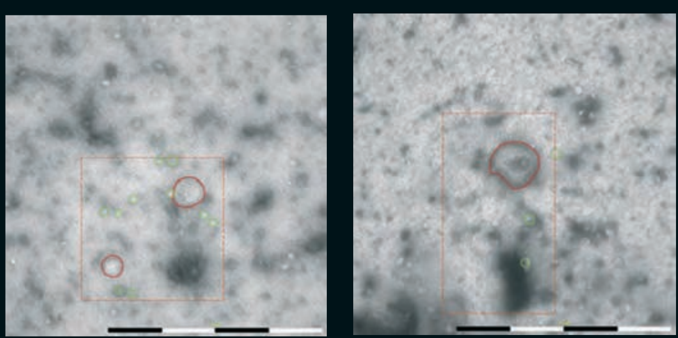
Images are automatically acquired in the selected areas

Particles with similar characteristics are grouped into distinct classes

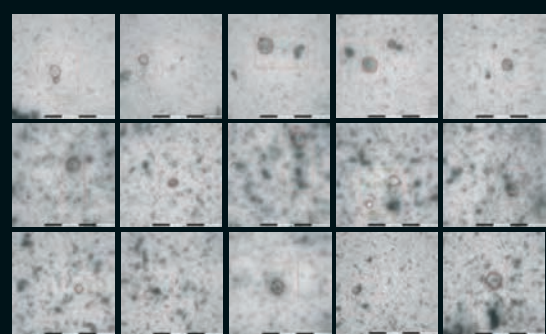
Quantative analysis of particle classes

Training data

Two examples (out of 15 in total)



Training data, complete set



■ LENTI

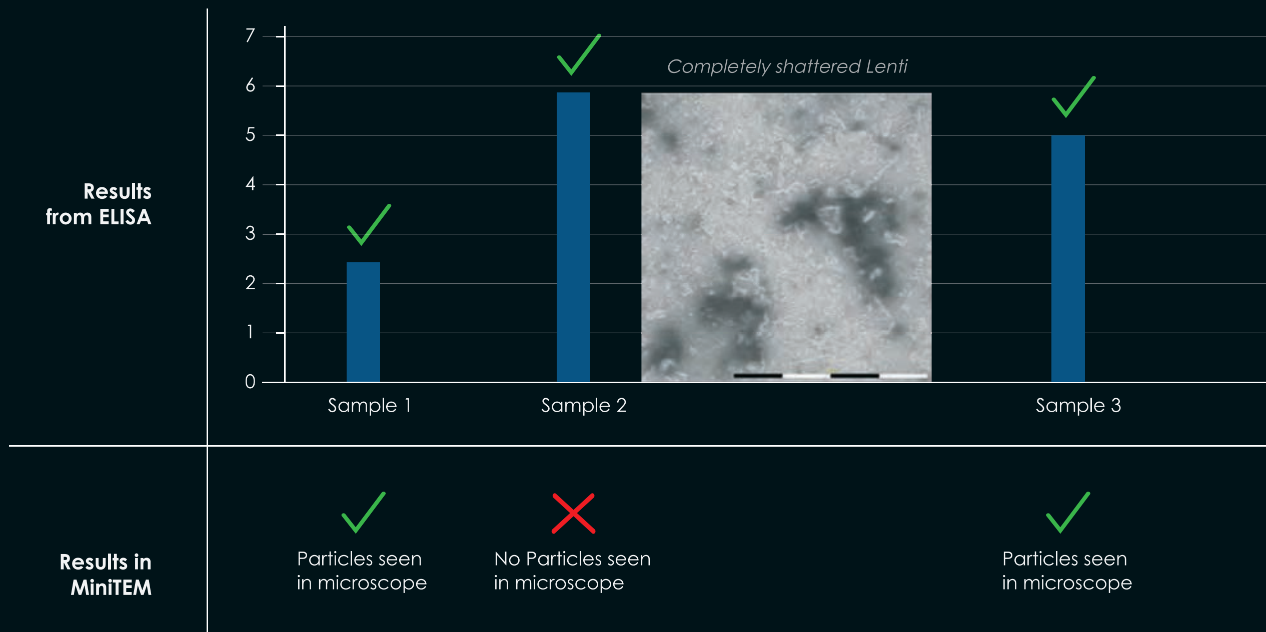
■ VESICLES

MiniTEM analysis can after training on training data be performed on Lenti samples.

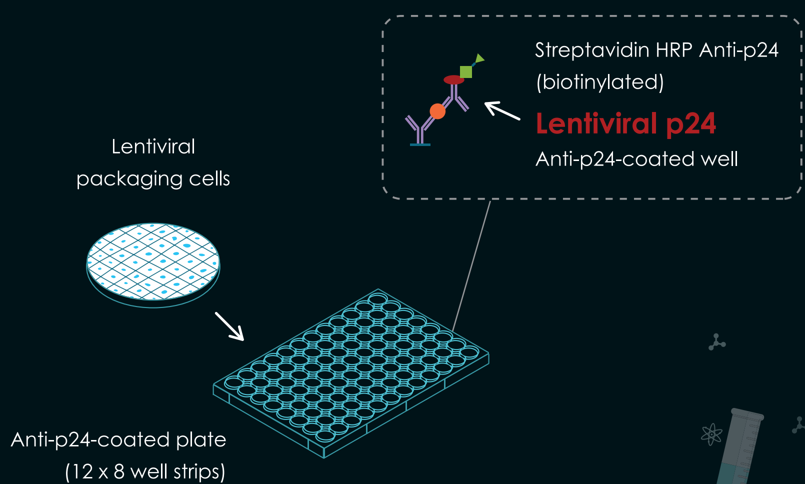
INDIRECT METHODS CAN BE HIGHLY INACCURATE

05.

Particles according to ELISA method



ELISA does not provide complete information of what is going on in your sample.
As shown in sample 2 a high concentration of P24 was detected but the MiniTEM analysis revealed that there were no intact Lenti particles present.

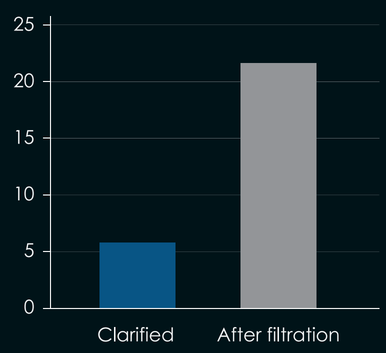


AFTER FILTRATION

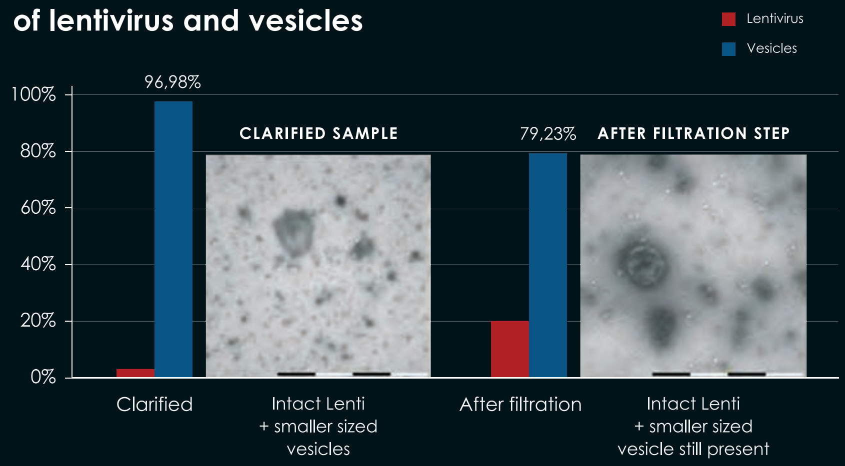
06.

Results after filtration

Number of lentiviruses per sample



Portion of on grid area of lentivirus and vesicles



SUMMARY & CONCLUSIONS

Automated imaging in MiniTEM combined with AI constitute powerful tools for characterizing complex samples such as Lentivirus.

The analysis provides valuable information about the effect of purification steps.

For this specific Lenti analysis we can conclude that the down-stream purification steps:

- In some cases affect the integrity of the Lenti particles
- Were able to enrich the Lenti particles
- Were not able to purify away the smaller sized vesicles

The ELISA analysis results probably stem from both attached and “free” p24.

For more info visit:

www.vironova.com