

Supplementary information

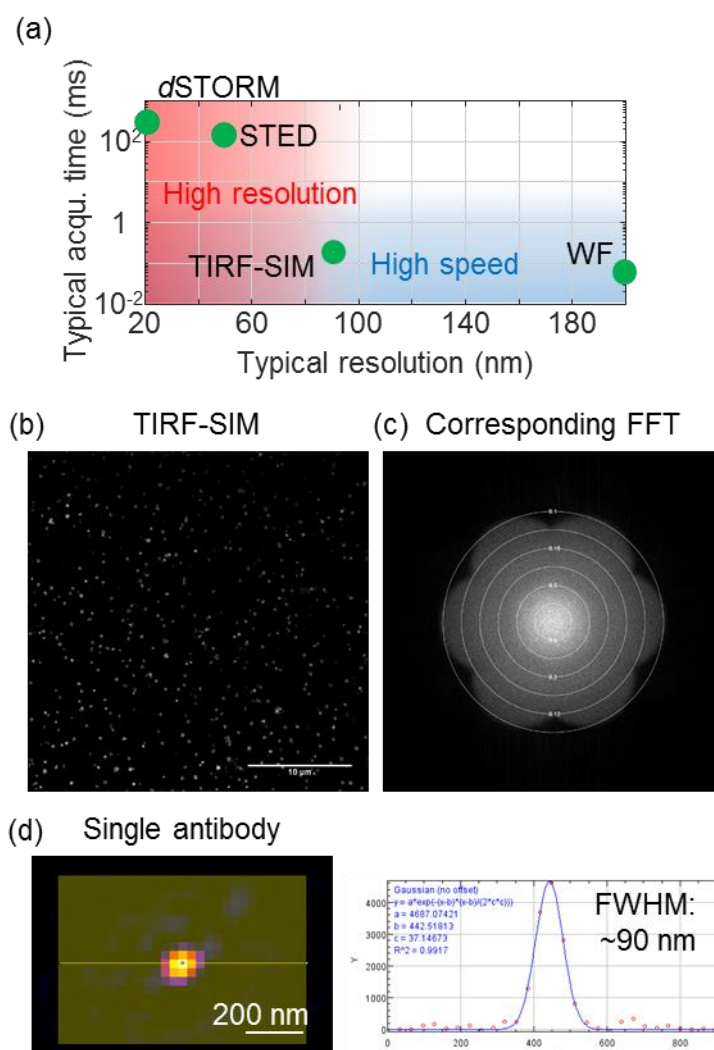


Figure S1: Resolution in SRM. (a) Typical spatial resolution and acquisition times for the imaging of NDV structures highlighting the trade-off between speed and resolution (b) Representative TIRF-SIM image obtained from purified B Victoria LAIV and its corresponding Fourier transform (c). The Fourier transform highlights the resolution ~90 nm. The plot was obtained using the SIMcheck plugin²⁹. (d) Image and cross section of a single secondary antibody labelled with DyLight 488, showing a FWHM of ~90 nm. FFT: Fast Fourier transform.

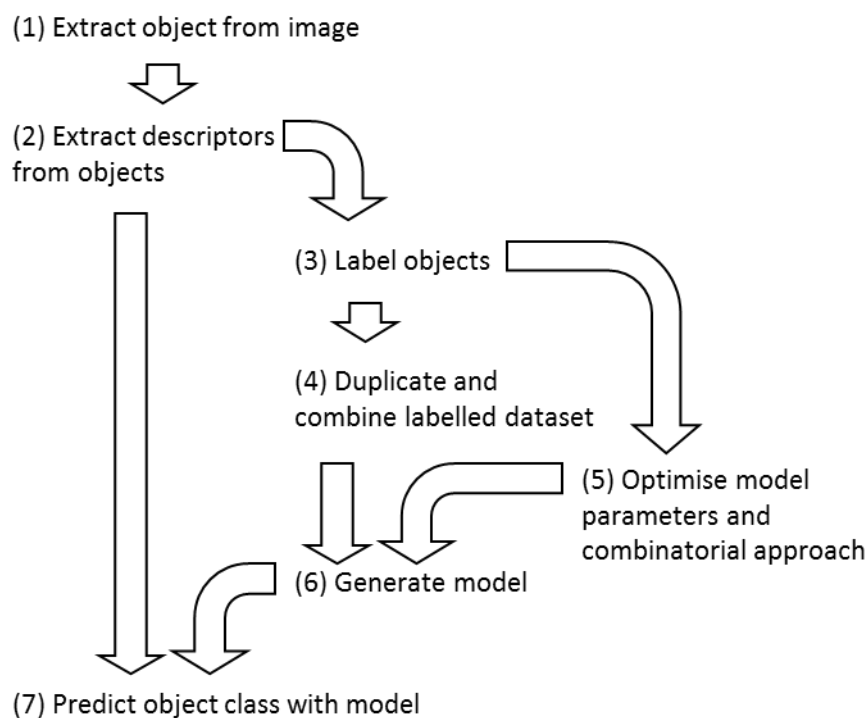


Figure S2: Flowchart describing the machine learning pipeline used here for image classification. A strong emphasis should be put on the choice of descriptors and the quality of the manual annotation (training dataset) prior to classification, as this will largely determine the quality of the classification.

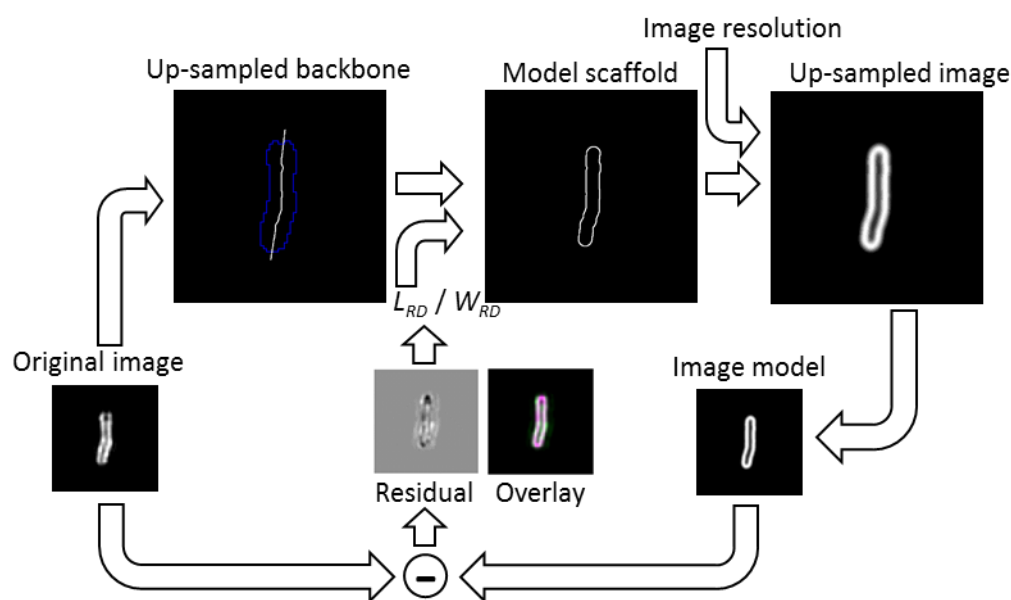


Figure S3: Image model for the analysis of the rod-shaped particles. The original image is segmented and thinned to obtain the backbone of the particle. The backbone is up-sampled and interpolated outside the particle. It is then used to compute the model scaffold. For this, each end of the backbone is independently grown or reduced to adjust its length, hence $L_{RD} = L_{tot} - L_1 - L_2$, where L_{tot} is the maximum length of the extended backbone, and L_1 and L_2 are the adjusted distances by which the backbone length is adjusted on each end respectively. Then, the image is dilated by a disk-shaped kernel of radius equal to half W_{RD} . The outline of this image gives the model scaffold. The scaffold is then convolved with a Gaussian kernel

representing the effect of image resolution (here 90 nm) and the image is down-sampled again to the original image size. The optimal L_{RD} and W_{RD} are those that minimize the difference image and the χ^2 .

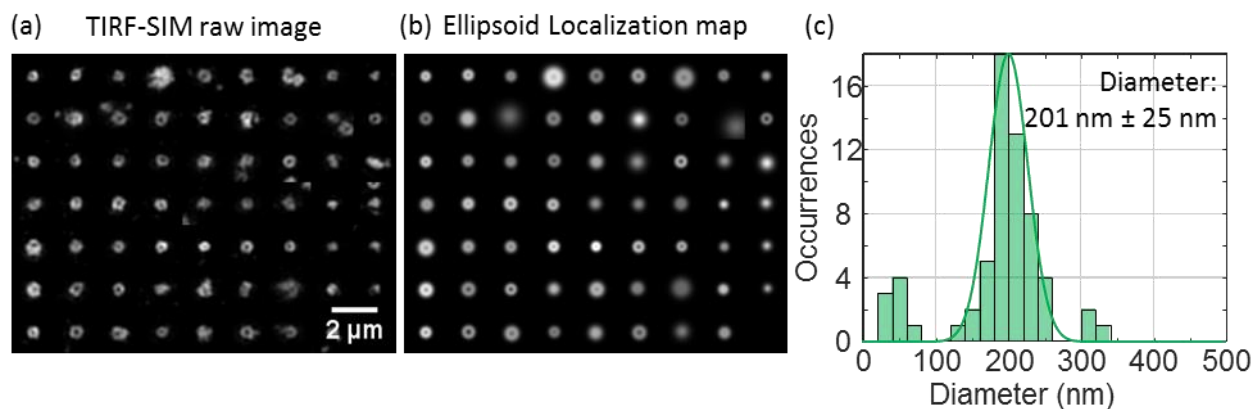


Figure S4: Spherical ELM analysis of the B. Victoria LAIV virus. Raw SIM images (a) and ellipsoid localization map (b). The distribution of diameters and a Gaussian fit are shown in (c). The diameter and error on the fit are the mean diameter and standard deviation obtained from the Gaussian fit.

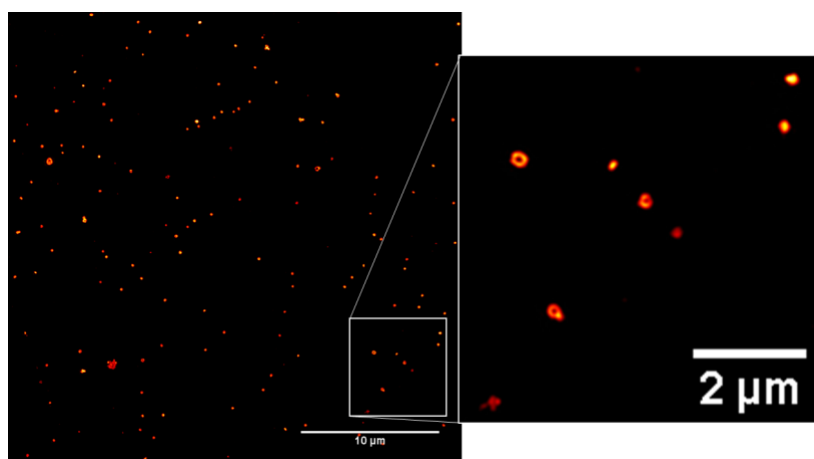


Figure S5: TIRF-SIM images obtained from pool harvested fluid (PHF). The images acquired here using PHF show an identical image quality as with highly purified samples.