



# Developing new diagnostic tools: understanding bovine viral infection mechanism

## Challenge

### Summary

The Nanoimager platform allows visualization, tracking and quantification of viral particles at a single-particle level, in solution and in live cells.

This type of research enables assessment of:

- Virus size and characterization to understand infectious agent life cycles
- Particle distribution in the infected host cells
- The infection mechanism
- Virus replication and envelopment within the host cell
- New therapeutic drugs and diagnostics tools
- Vaccine development for infectious diseases prevention

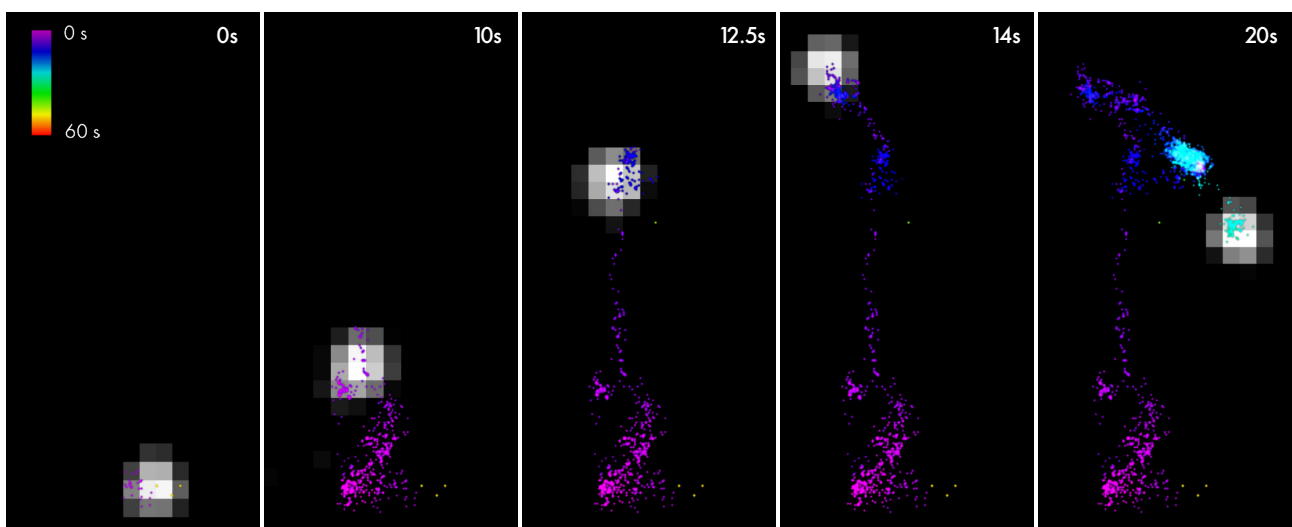
Bovine herpesvirus-1 (BHV-1) is known to cause several diseases worldwide and, even though mortality rates are low, these diseases have significant impact on animal growth, milk production and livestock well-being. Finding efficient ways of treating viral infections can be improved by gaining insights into BHV particle structure, transmission mechanisms, and drug-targeted events of its life cycle.

One of the main challenges in understanding viral spread and infection of host cells is their small size – viral particles range from a few micrometers down to nanometers. These often appear as diffraction limited spots in conventional light microscopes and, to date, electron microscopy was the technique of choice in viral particle research.

The Nanoimager, thanks to its single-particle tracking (spt) imaging capabilities, can effectively address this challenge by visualizing viral particles and their contents at a single-molecule level in solution and in living host cells. Viral particles can be imaged in real time, with 4 different laser lines, providing highly flexible sample labelling options.

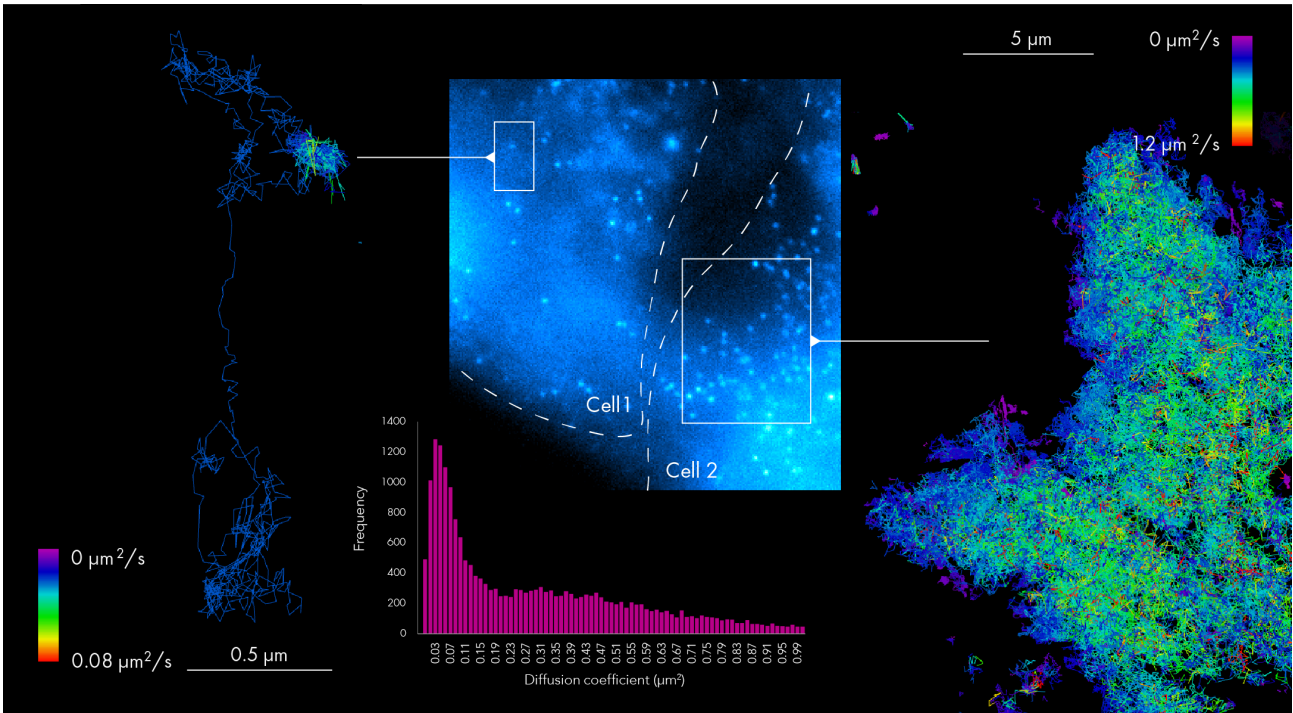
## Results

The Nanoimager is a super-resolution microscope that enables the visualization of single particles with simultaneous single-molecule dual color imaging. Figure 1 shows single-particle tracking of mCherry-labelled BHV1 in live bovine fibroblast cells. The experiment aimed to characterize the dynamic localization of viral particles throughout their life cycle: assembly and maturation in the cell nucleus, envelopment and exocytosis from the cell as mature virions. Viral particle localizations were identified and colored-coded according to their appearance over time (Figure 1). Representation of this data enables spatio-temporal characterization of viral infection at a single-particle level.



**FIGURE 1** Single-particle tracking of mCherry-labelled BHV1 virus in live cells. Single particle localizations were identified over time and colored-coded according to the time of appearance.

Subsequently, the dedicated NimOS software tracking tool was used to depict particle trajectories in a whole field of view and tracks were colored according to their diffusion coefficients. The viral particles in cell 1 showed lower diffusion coefficients and longer trajectories than viruses within cell 2. Representation of the diffusion coefficient distribution for the whole field of view showed two peaks at approx.  $0.05 \mu\text{m}^2/\text{s}$  and  $0.31 \mu\text{m}^2/\text{s}$ , indicating the presence of two different populations of particles (Figure 2).



**FIGURE 2**

Diffusion coefficient distribution of single mCherry-labeled BHV1 particles in bovine fibroblasts. Particles in cell 1 were characterized by a lower diffusion coefficient than those in cell 2. Sample prepared by Dr. M. Rychlowski, Intercollegiate Faculty of Biotechnology UG & MUG, Gdansk, Poland.

## Solution With The Nanoimager

The Nanoimager provides a comprehensive solution to develop better diagnostic tools, vaccines and antiviral treatments by making studies of infectious agents easy and accessible to all. Its compact size and unrivalled stability make it a strong choice for BSL3 safety cabinets and BSL4 facilities. The capability to track and quantify behaviors of tens to thousands of particles with immediate access to quantified information provides new insights into our knowledge of these previously poorly characterized particles and speeds up the process of discovery in this field.

To learn more about the microscope features, its different applications and ONI visit [www.oni.bio](http://www.oni.bio).



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