Nanoimager

& viral particle research

A versatile platform for viral particle research

Viral particles vary greatly in size, ranging from approximately 20 to 300 nm in diameter, which is smaller or close to the theoretically achievable resolution of a conventional light microscope. Consequently, there are many mechanistic and functional characteristics of viral particles that are yet to be elucidated.
Recently developed super-resolution techniques, such as PALM and dSTORM, overcome the diffraction limit of light and allow for viruses and their structure to be studied at single-molecule resolution.

The Nanoimager is the world's first desktop-compatible microscope able to easily visualize viral particles with 100x magnification and a resolution reaching 20 nm. Importantly, it uses ultra-high single-molecule sensitivity to track single viral particles in fluorescence mode, allowing sizing and counting using tracking-based methods. The ability to visualise two colors simultaneously enables researchers to follow fluorescently labeled viral particles in live cells, in real time. Additionally, the Nanoimager's unique, compact design makes it an ideal tool to support research performed in space limited laboratories, including BSL3 safety cabinets and BSL4 facilities.

Visualizing viral particles with exceptional resolution

Super-resolution imaging can be used to study fine morphological details and the precise localization of viral particles. Figure 1 provides an excellent example of the application of dSTORM to investigate viral particles. It shows a coverslip-adhered reconstructed HIV particle, in which the surface glycoprotein and cellular host factors incorporated into the particle have been labeled with antibodies conjugated to AF647 (red) and AF488 (green), respectively. The estimated size of this example viral particle is approximately 194 nm, as judged by the surface glycoprotein distribution (in red).

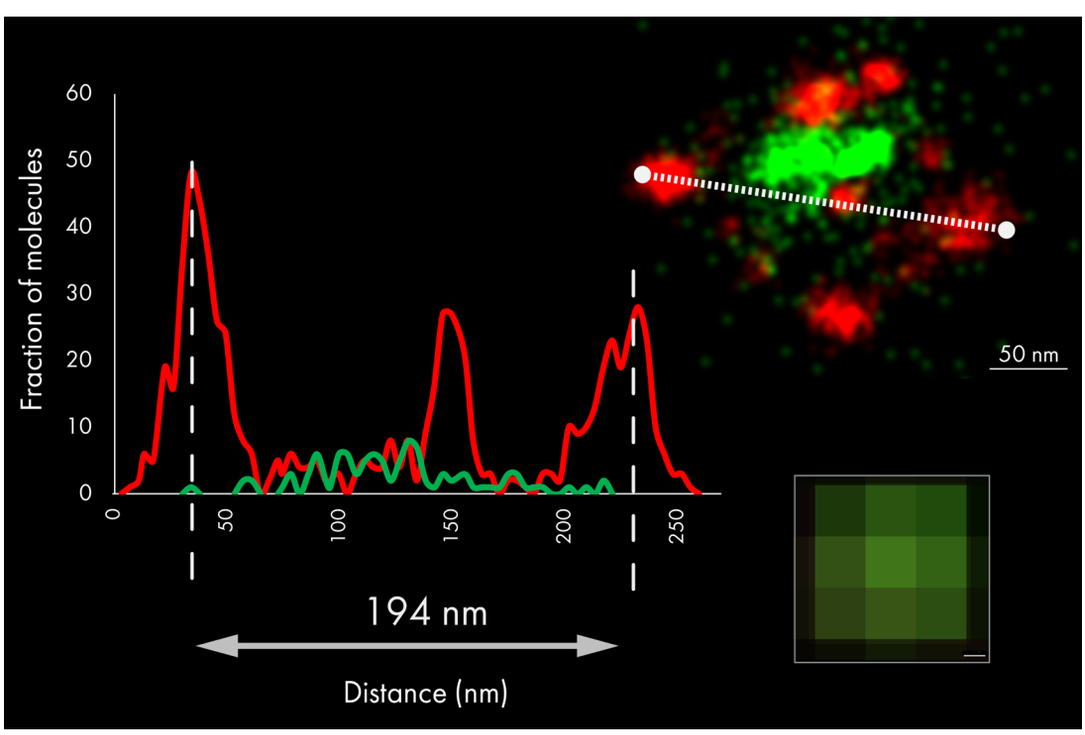

FIGURE 1
Reconstructed HIV particle image acquired by dSTORM. The image reveals the spatial organization of surface glycoproteins and cellular host factors incorporated into the particle, labeled with antibodies conjugated to AF647 (red) and AF488 (green), respectively. Inset: a diffraction-limited wide field image of the viral particle, scale bar: 50 nm. Sample prepared by Dr. G. Melikian's Lab, Emory University, Atlanta.

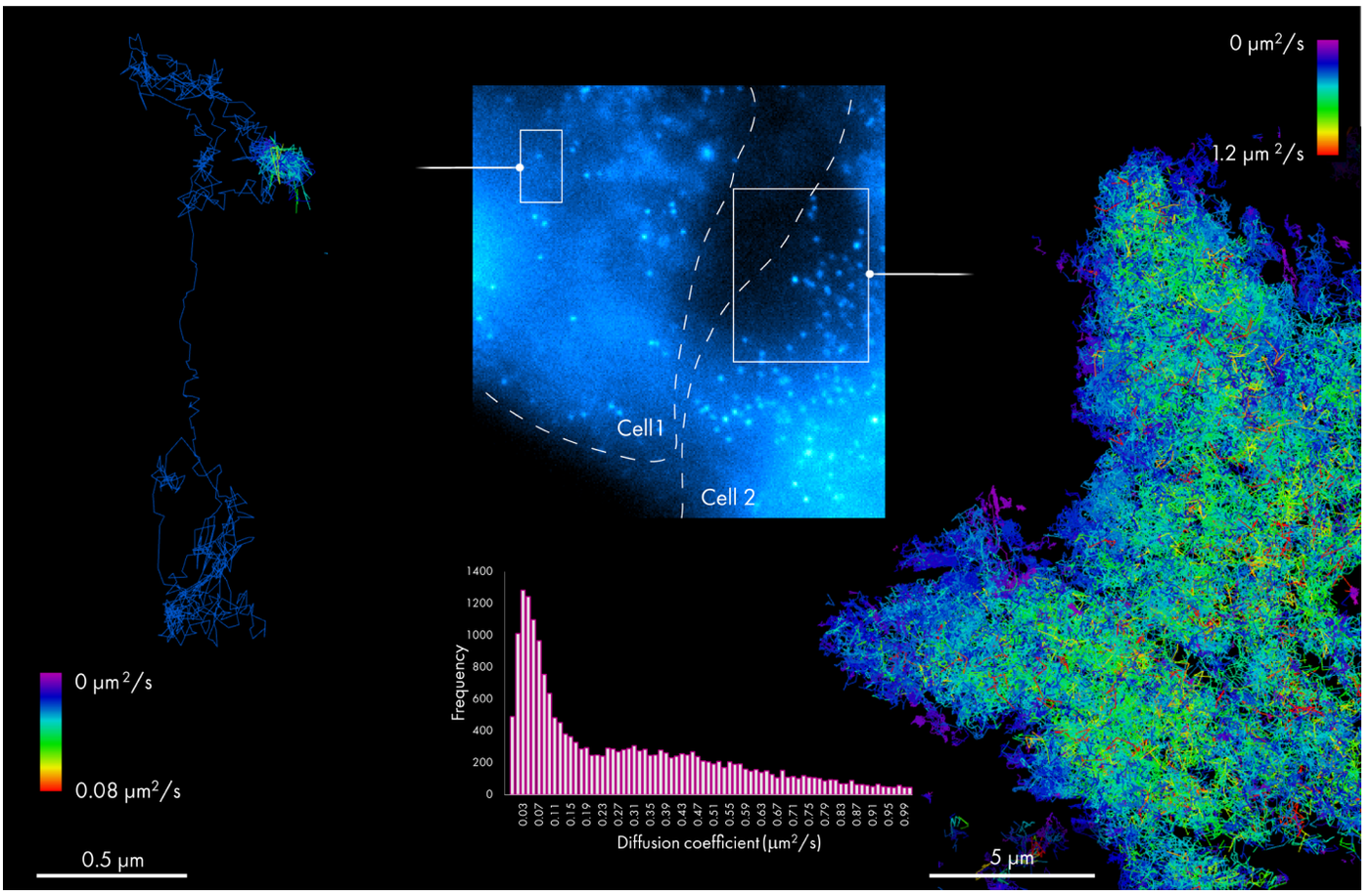

FIGURE 2

Integrating visualization with tracking, sizing and counting

Besides imaging cellular ultrastructure in high resolution, the Nanoimager can track single molecules and particles in purified samples in solution, as well as in live cells. This enables spatio-temporal dynamic localization of viral particles, e.g. during assembly and maturation, envelopment and exocytosis as mature virions. Figure 2 presents an example of BHV1 (Bovine Herpes virus 1, labeled with mCherry) tracking in bovine fibroblasts. The particles in cell 1 have lower diffusion coefficients than those in cell 2. This may indicate, that virions in cell 2 are at the later stage of their development. The experiment was run in order to determine the number, diffusion coefficients and directionality of BHV1 particles throughout their life cycle.

SIM & Confocal

New imaging modalities were recently added to the Nanoimager: confocal and Structured Illumination Microscopy (SIM). These techniques allow to achieve an axial resolution up to 2 times better than a wide-field microscope using commonly available fluorophores. SIM and confocal are useful when studying morphological changes and sub-cellular structures in live cells, e.g. upon exposure to infectious viruses or in time-lapse series. An example of a SIM image is presented in Figure 3. To further support live cell imaging, the Nanoimager is equipped with whole body heating and it is compatible with microfluidics.

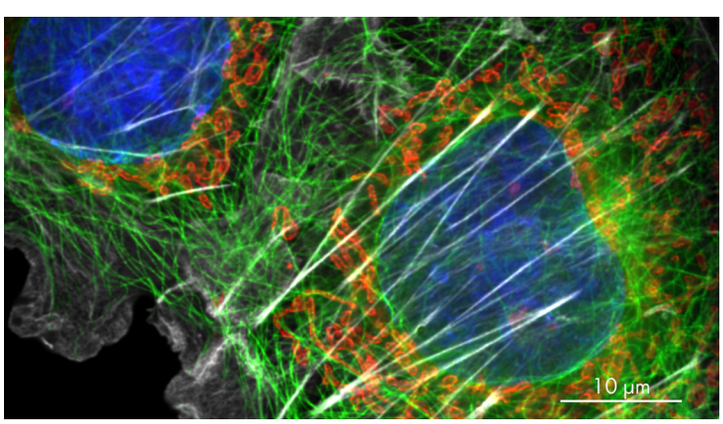

FIGURE 2 (TOP)

BHV1 tracking in bovine fibroblasts. The presented tracks are colored according to their diffusion coefficient and the viral particles were labeled with mCherry fluorescence protein. Chart shows distribution of diffusion coefficients for the whole field of view. Sample by Dr. M. Rychlowski, Intercollegiate Faculty of Biotechnology UG&MUG, Gdansk, Poland

FIGURE 3 (LEFT)

SIM image of Cos7 cells stained with TOM20 (mitochondria marker, red), DNA (DAPI, blue)and SiR (actin, grey) overlayed with confocal image of tubulin (microtubules, green).

About us

We believe our future depends on cutting edge scientific discovery.

Our mission is to positively impact
people's lives by enabling innovation across life sciences,
medicine and beyond, so that we support those who
seek answers to some of the world's biggest problems.

ONI is focused on removing barriers to make
science more effective, accessible, affordable.
We are creating the ultimate science ecosystem
that could one day be used by anyone, anywhere from the
research bench to your doctor's office.

e: hi@oni.bio • t: +44 (0) 1865 951 820
ONI, Linacre House, Jordan Hill Road, Oxford, OX2 8TA, UK
WWW.ONI.BIO

@oniHQ

@ONI (Oxford Nanoimaging)