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IMAGING TECHNIQUES FOR STUDYING VIRUS–CELL INTERACTIONS: A REVIEW OF CURRENT METHODS AND CHALLENGES

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Abstract. Knowledge of virus-host cell interactions is central to the formulation of antiviral therapies and vaccines. Because of their nanoscale size and dynamic nature, viruses are inherently difficult objects to investigate. Virus characterization, such as imaging viral structures, intracellular viral trafficking, and infection molecular mechanisms, has relied heavily on sophisticated imaging approaches. Classical light microscopy imaging, such as fluorescence and super-resolution microscopy, provides information on viral entry, replication, and protein localization within living cells. Electron microscopy (EM) techniques, such as Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM), and Cryo-Electron Microscopy (Cryo-EM), provide high-resolution structural information on the viruses and their replication compartments. Advances in correlative imaging techniques, which include light and electron microscopy, have improved our ability to study virus-induced cellular changes in three dimensions. But in comparison to the earlier developments, it remains challenging in virus imaging: a compromise between resolution and sample preparation, restrictions in labeling methods, the challenge in imaging rapid virus-host interactions, and biosafety limitations for highly pathogenic viruses. Solutions to these types of issues will be provided with the newer techniques such as AI-powered imaging analysis, nanotechnology-based imaging probes, and cryo-electron tomography. This review covers the present imaging methods in virology, their utilities and limitations, as well as future prospects, with an emphasis on microscopy to discern the interaction of viruses with cells electron microscopy.

Key words: *molecular mechanisms, transmission electron microscopy, virus, imaging techniques, cryo-electron tomography, microorganisms.*

МЕТОДЫ ВИЗУАЛИЗАЦИИ ДЛЯ ИЗУЧЕНИЯ ВЗАИМОДЕЙСТВИЙ ВИРУС–КЛЕТКА: ОБЗОР СОВРЕМЕННЫХ МЕТОДОВ И ПРОБЛЕМ

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Резюме. Знание взаимодействия вируса и клетки-хозяина имеет решающее значение для разработки противовирусной терапии и вакцин. Из-за своего наномасштабного размера и динамической природы вирусы являются сложными объектами для исследования. Характеризация вирусов при помощи визуализация вирусных структур, внутриклеточного вирусного трафика и молекулярных механизмов инфекции, в значительной степени опиралась на сложные технологические подходы. Классическая световая микроскопия, такая как флуоресцентная микроскопия и микроскопия сверхвысокого разрешения, предоставляет информацию о про-

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никновении вируса, репликации и локализации белка в живых клетках. Методы электронной микроскопии (ЭМ), такие как просвечивающая электронная микроскопия (ПЭМ), сканирующая электронная микроскопия (СЭМ) и криоэлектронная микроскопия (крио-ЭМ), предоставляют структурные данные высокого разрешения о вирусах и их репликационных компартментах. Достижения в области корреляционных методов визуализации, которые включают световую и электронную микроскопию, улучшили возможность изучать вызванные вирусом клеточные изменения в трех измерениях. Но по сравнению с более ранними разработками, визуализация вирусов остается сложной из-за компромисса между разрешением и подготовкой образцов, ограничений в методах маркировки, проблемы визуализации быстрых взаимодействий вируса с хозяином и ограничения биологической безопасности для высокопатогенных вирусов [9]. Решения таких проблем будут предоставлены с помощью новых методов, таких как анализ изображений на основе ИИ, зондов для визуализации на основе нанотехнологий и криоэлектронной томографии. В настоящем обзоре рассматриваются современные методы визуализации в вирусологии, их применимость и ограничения, а также будущие перспективы с акцентом на микроскопию для распознавания взаимодействия вирусов с клетками [50].

Ключевые слова: *молекулярные механизмы, просвечивающая электронная микроскопия, вирус, методы визуализации, криоэлектронная томография, микроорганизмы.*

Introduction

Viruses are obligate intracellular parasites requiring host cells to multiply. Understanding how viruses communicate with their host cells — isolate, multiply, form, and exit is very essential for the development of antiviral drugs and vaccines. As viruses on the move are detectable only with the aid of advanced imaging techniques due to their nanometer-scale and dynamic character, the techniques permit researchers to trace viral structures, track virus mobility inside cells, and determine the molecular mechanism of virus–cell interaction [7].

In the last decades, microscopy has transformed the field of virology, providing unparalled information on the viral life cycle. Electron microscopy had been the force that changed the field with its arrival in the early decades of the 20th century and the provision of the first viral particle images [23]. Light microscopy, electron microscopy, and correlative imaging methods, in turn, are still under continuous development since then, allowing researchers to visualize viruses more vividly and even in real-time. In the current years, the use of super-resolution microscopy, cryo-electron tomography, and single-molecule imaging has become instrumental in elucidating fine-scale molecular mechanisms of virus infection [8].

Despite these advances, virus imaging remains challenging. Most viral mechanisms are in the nanometre range, which is below the resolution limit of standard light microscopy. Moreover, the imaging of live viruses inside infected cells requires techniques that balance resolution, speed, and sample minimization damage. This review shall detail current imaging methods utilized in virology, their usage, their strengths, and their weaknesses, as well as the future direction of viral imaging.

While there are variations, all viruses adhere to basic steps: entry into host cells, replication of the genome, assembly of new virions, and their subsequent release. Techniques such as confocal microscopy, imaging flow cytometry, and cryo-electron microscopy are routinely utilized in virology to visualize and investigate such interactions between host cells and viruses [28].

Virus-Cell Interactions: A Detailed Overview

Viruses are obligate intracellular parasites that depend entirely on the host cells for their growth and survival. Virus-cell interactions include several steps, each necessary for the completion of the viral life cycle: attachment and entry, penetration and uncoating, replication and gene expression, virus assembly and maturation, and virion release and spread. Each step has some molecular mechanisms that vary with the type of virus, structural make-up, and the host cell it infects [36].

Attachment and Entry: The First Step in Viral Infection

Infection begins with attachment through interactions between viral surface proteins and specific receptors on a cell membrane. Binding specificity defines tropism and host range for a virus. HIV binds on CD4 receptors, and the influenza virus bind on sialic acid residues. Certain viruses also require additional co-receptors. HIV, for example, requires CCR5 or CXCR4 as a co-receptor with CD4. Efficiency at infection also relies on number and density of viral receptors on a cell surface, as increased receptor density increases virus attachment possibilities [38].

Entry mechanisms vary: enveloped viruses use membrane fusion or receptor-mediated endocytosis; non-enveloped viruses use pore formation or endocytosis [2].

Penetration and Uncoating: Delivering the Viral Genome

Upon infection of the cellular host, the virus must release its genome for replication through a process called uncoating. Uncoating occurs via membrane

fusion or through endocytic vesicles. Non-enveloped viruses cause endosomal damage to deliver their genome into cytoplasm. Low endosomal pH can trigger a conformational shift in viral proteins for penetration through the membrane [19].

Replication and Gene Expression: Hijacking the Host Machinery

After uncoating, the viral genome must be replicated and transcribed to generate viral proteins. Replication is different based on the nature of the viral genome, which can be DNA or RNA, single-stranded or double-stranded, positive-sense or negative-sense. DNA viruses replicate in the nucleus using host or viral DNA polymerases to replicate new genomes. RNA viruses, however, usually replicate in the cytoplasm, as they require RNA-dependent RNA polymerases (RdRp) to replicate new RNA strands. This enzyme is not present in host cells, and therefore RNA viruses must encode it in the genome.

Positive-sense RNA viruses, can be translated as soon as they are in the cytoplasm, while negative-sense RNA viruses, must form complementary positive-sense RNA before they can be translated. Retroviruses, use a unique strategy in which reverse transcriptase (RT) copies their RNA genome into DNA, which then becomes integrated into the host genome, allowing long-term replication and persistence [36].

Viral protein translation is another process that has been maximized by viruses for successful replication. Certain viruses produce a single large polyprotein, which is subsequently cleaved into functional proteins, while others use processes like alternative splicing, ribosomal frame-shifting, and leaky scanning to produce more than one protein from a single mRNA transcript. Most viruses repress host cell protein synthesis to promote their gene expression.

Virus Assembly and Maturation: Constructing New Virions

After replication and protein synthesis, the new viral proteins and genomes must be packaged into mature virions. Virus assembly is a highly organized workflow that occurs in specialized areas of the host cell. Certain viruses, such as herpesviruses and adenoviruses, are assembled within the nucleus, and others, such as coronaviruses and flaviviruses, are assembled within the cytoplasm. Certain viruses, such as HIV, are assembled at the plasma membrane, where they acquire their envelope through budding [19].

Maturation is an essential process of viral assembly because some viruses require post-assembly processing to become infectious. HIV virions, for instance, are proteolytically cleaved by viral protease within their Gag polyproteins, allowing infectious particle formation. Prevention of this occurrence is the mode of action of protease inhibitor drugs for antiretroviral therapy.

Virion Release and Spread: Escaping the Host Cell

The final process in the viral life cycle is the release of new virions to propagate the infection. Non-enveloped viruses exit by lysis, where they burst the plasma membrane and destroy the host cell. Enveloped exit by budding, gaining their envelope from the host cell membrane in the process. Some viruses, such as coronaviruses and Hepatitis B, are released by exocytosis, where virions are enclosed within vesicles and transported to the plasma membrane for secretion [54].

Viruses have developed other mechanisms of spread within the host. Others, like measles virus, induce syncytium formation by inducing infected cells to fuse with neighboring cells and become multinucleated giant cells, facilitating cell-to-cell spread. Others, like Vaccinia virus, use actin-based motility to move from cell to cell without being exposed to the extracellular environment.

Why Imaging is Crucial for Studying Virus-Cell Interactions?

Virus-cell interactions are vital to comprehend in virology, as these determine the entire virus life cycle from entry into the host cell, through replication and eventual release. Imaging techniques have become indispensable probes to study such processes, and they offer images of how the viruses infect the host cells, take over the cellular machinery, and spread [55].

Due to the nanoscale dimension of viruses, traditional biochemical and molecular methods fall short in unveiling the structural and dynamic characteristics of viral infection. High-resolution imaging methods, such as Light Microscopy-Based Methods, Electron Microscopy (EM) Methods, Correlative and Multiscale Imaging, and Molecular and Functional Imaging, allow researchers to witness these intricate interactions with unprecedented accuracy. Here, we shall elaborate on each of these techniques in detail, their concepts, uses, and how they have contributed to virus–cell interaction understanding [33].

Imaging Techniques for Studying Virus-Cell Interactions

Light Microscopy

Light microscopy has long been the workhorse of biological studies, allowing researchers to see cell structure and processes with precision. In virology, such approaches serve particular application for the examination of virus–cell interactions, with information on viral entry, replication, assembly, and egress. Visualization of the ability of the virus of investigation actually taking place under real-time conditions of cells has redefined understanding of the mechanism of infection and host-pathogen interactions [46].

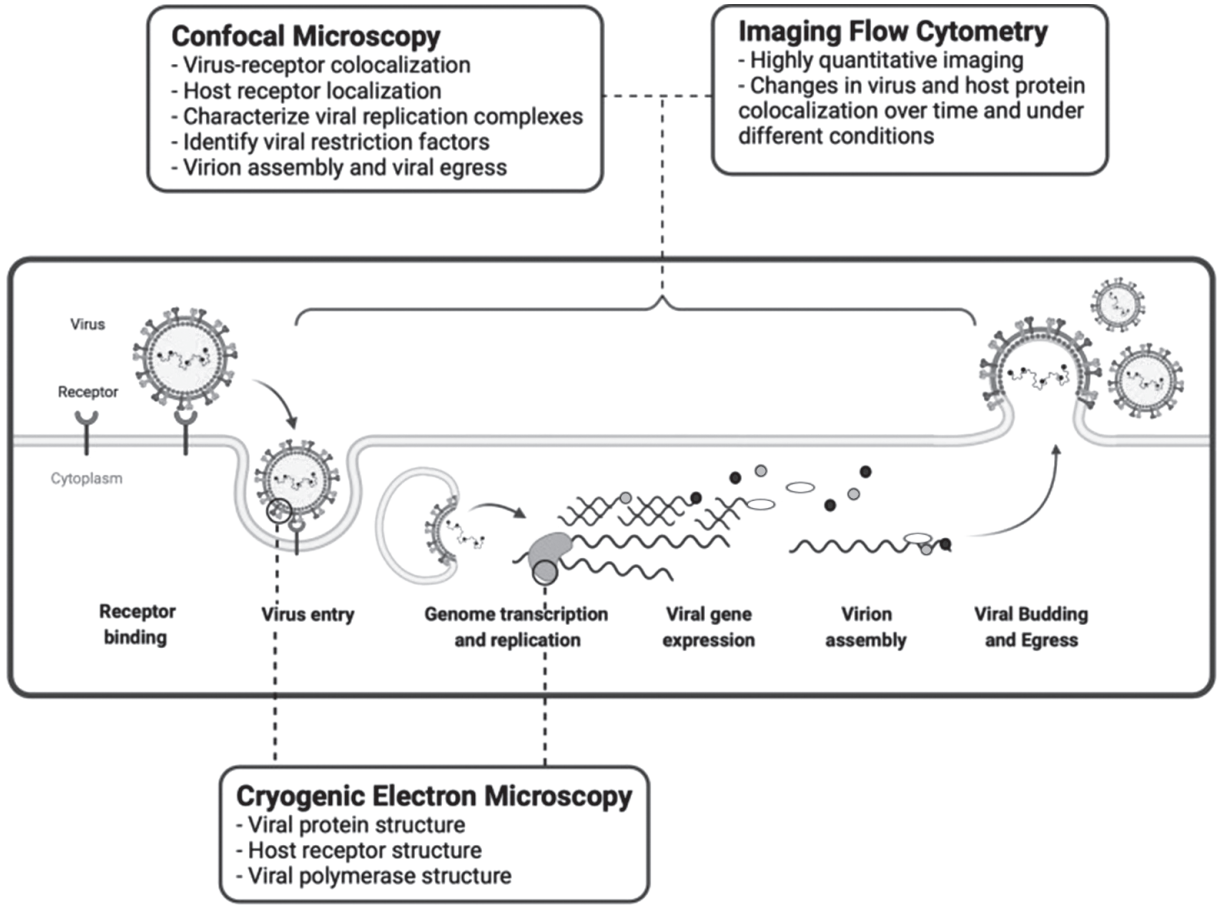

Figure. Imaging methods utilized to investigate virus-host interactions throughout the viral life cycle
Note. The illustration presents the RSV, a negative-sense single-stranded RNA (–ssRNA) enveloped virus, replication cycle as a model.

Recent advances of light microscopy have collectively enhanced the imaging depth, contrast, and the spatial resolution, allowing researchers to bypass the diffraction limits of conventional optical systems. By using the application of fluorescence labeling and the newer imaging modalities, the viral constituents can be imaged at the molecular level, providing unprecedented insights into their functional dynamics and the structural organization [31]. These procedures capitalize on the principles of light, such as refraction and diffraction, for the generation of high-resolution images of the virus-host interaction, providing critical information for antiviral research as well as for therapeutic discovery.

Super-Resolution Microscopy: STED, STORM, and PALM

Microscopy Super-resolution microscopy techniques have revolutionized cellular imaging by overcoming the diffraction limit of conventional optical microscopy of approximately 200 nm in the lateral plane and 900 nm in the axial plane. Abbe's principle limits conventional fluorescence microscopy such that the minimum resolvable feature is determined by the wavelength of the illuminating light and the numerical aperture of the imaging apparatus. Super-resolution microscopy techniques circumvented this limit based on different strategies to achieve nanoscale imaging. Some of these include Stimulated Emission Depletion (STED), Stochastic Optical Reconstruction Microscopy (STORM), and Photoactivated Localization Microscopy (PALM), which are some of the standard techniques to achieve 10–50 nm resolutions [44].

Stimulated Emission Depletion (STED) Microscopy

STED microscopy is a directed super-resolution imaging technique bypassing diffraction limit in traditional fluorescence microscopy by stimulated emission depletion. It was initially described by Stefan Hell and Jan Wichmann in 1994 [15]. The mechanism relies upon two lasers: a pumping excitation laser inducing fluorophores into a state of excitement, a second doughnut-shaped laser beam de-excitating fluorophores in a region outside the focal area of interest by stimulated emission, effectively shrinking the effective point spread function [32].

The most striking advantage of STED microscopy comes from its capability of reaching resolutions of as low as ~70 nm, demonstrated from intravital microscopy of mouse brain tissue. This has made STED microscopy highly valuable in virus-host interaction research, where the technique has been used in imaging viral infection cellular structures at

higher resolution [41]. STED microscopy has several disadvantages, however, such as relatively strong laser intensities, resulting in photochemical bleaching and phototoxicity. Solutions have been aimed at optimizing the technique using reversible saturable/switchable optical linear fluorescence transition (RESOLFT), allowing lower laser intensities while with no loss in resolution [34, 63].

Stochastic Optical Reconstruction Microscopy (STORM)

STORM is a stochastic super-resolution method predicated on the exact localization of individual fluorescent molecules for reconstructing an image at the nanoscale [43]. While STED makes use of structured illumination, STORM accomplishes super-resolution by stochastically activating a sparse number of fluorophores in such a manner that merely a few molecules are in the fluorescent state at a particular instant of time. Consecutive thousands of images are gathered, and the exact locations of individual molecules are reconstructed computationally to construct a super-resolved image [57].

STORM has been widely applied in virology research, especially in the investigation of virus-host interactions. Single-molecule super-resolution STORM imaging, for instance, was employed to investigate CD4 redistribution in the plasma membrane of T cells upon HIV-1 binding, giving an insight into receptor organization at the nanoscale. It has enabled researchers to describe viral particle interaction with host cell receptors with unprecedented spatial accuracy [45].

Although high resolution is achieved with STORM, it necessitates high labeling densities and extended acquisition times and thus is less appropriate for live-cell imaging [20]. Furthermore, photobleaching of fluorophores can restrict imaging time, such that the utilization of photoswitchable dyes is necessary to compensate for signal loss [17].

Photoactivated Localization Microscopy (PALM)

Another single-molecule, single-molecule super-resolution technique, PALM achieves this imaging through the recruitment of the stochastically activated photoactivatable fluorescent proteins. Like STORM, PALM also relies upon the single-molecule precise localization through repeatedly imaging the low numbers of activated fluorophores [70].

The PALM has also been used extensively in cell biology and virology research, in a bid to study the assembly, release, and maturation of the virus. PALM imaging, in combination with other methods, has revolutionized the study of the structure of the virus at a nanoscale, for instance, in HIV-1, where PALM was used in combination with other imaging methods of high resolution [34].

Among the strengths of PALM is its compatibility with genetically encoded fluorophores, making it highly compatible with live-cell imaging [23]. Like STORM, however, PALM requires long image acquisition and computational post-processing, which frustrates its real-time applications. Despite these drawbacks, the technique is an extremely valuable tool for investigating dynamic viral and cellular processes at the molecular level [13].

Single-Molecule Imaging: smFRET and TIRF Microscopy

Single-molecule imaging techniques have revolutionized the viewing of dynamic molecular interactions in real time. These high-resolution techniques circumvent ensemble averaging by detecting and tracking single molecules, enabling researchers to examine intricate biological processes at previously unimaginable resolutions in space and time. Two such techniques that are at the forefront of this field are Single-Molecule Förster Resonance Energy Transfer (smFRET) and Total Internal Reflection Fluorescence (TIRF) Microscopy. These techniques have been instrumental in the progress of virus-host interaction studies, especially in investigating viral membrane fusion and protein conformational change [35].

Single-Molecule Förster Resonance Energy Transfer (smFRET) Imaging

Single-Molecule Förster Resonance Energy Transfer (smFRET) is a fluorescence microscopy that facilitates real-time imaging of biomolecular interactions on the nanometer scale. smFRET has been most applicable in virus research since it reveals dynamic conformational change in viral proteins, especially virus spike–host interactions [26].

Principle of smFRET is based on the Förster resonance energy transfer (FRET) mechanism, which describes non-radiative energy transfer between two fluorophores: a donor and an acceptor. The efficiency of energy transfer (E) is inversely proportional to the sixth power of the distance (R) between the fluorophores, following the equation:

$$E = 1 / (1 + (R/R0)^6).$$

This renders smFRET very sensitive to nanoscale molecular interactions, with distance measurements as low as 1–10 nm.

In viral research, smFRET has been used extensively to probe viral membrane fusion and spike protein dynamics. It has been particularly useful in the study of viral glycoprotein conformational changes that accompany entry into host cells. For example, smFRET has uncovered dynamic conformational states of the HIV-1 envelope (Env) protein and the SARS-CoV-2 spike protein during host interactions. The method has given an understanding of how viral proteins move through various structural states, e.g., pre-fusion and post-fusion states, which are essential in the development of antiviral therapies [35].

Total Internal Reflection Fluorescence (TIRF) Microscopy

Total Internal Reflection Fluorescence (TIRF) microscopy is an extremely advanced imaging technique for biomolecules with high resolution near

the plasma membrane. It is particularly suitable to investigate cell surface interaction and membrane-bound processes, e.g., endocytosis, exocytosis, and viral entry [45].

TIRF Microscopy relies on the principle of total internal reflection, whereby the incident laser beam hits the interface between two media of different refractive indices, e.g., glass and water. Total internal reflection happens at an angle of incidence larger than a certain critical angle and creates an evanescent wave that penetrates only an infinitesimal distance (~100 nm) into the sample. The wave excites fluorophores at the interface, minimizing background fluorescence and improving signal-to-noise ratio [12].

TIRF microscopy has seen extensive application in the study of viral interaction with the cell membrane. TIRF microscopy has been in wide use in the imaging of dynamic processes, which include virus entry and host cell interaction. TIRF has also been combined with single-molecule fluorescence methodologies, such as smFRET, to investigate real-time viral spike protein conformation dynamics during virus-host interaction. Together, they have enabled the HIV-1 and SARS-CoV-2 receptor-binding events to be investigated in depth to reveal viral membrane fusion and antibody neutralization mechanisms [21].

TIRF microscopy provides key benefits to live-cell imaging in that it restricts fluorescence excitation to a thin plane (~100 nm) near the coverslip, reducing background noise and phototoxicity. This provides a method well-suited for imaging activity at the plasma membrane, including receptor-ligand interactions and endocytosis. TIRF is restricted, however, in imaging deeper cellular structures since the evanescent wave only penetrates a short distance from the coverslip [60].

Fluorescence Microscopy

Fluorescence microscopy is a sophisticated imaging method that utilizes light emitted by fluorophores to follow particular molecules and cellular structures. The method has transformed biological research by enabling precise probing of cellular processes with improved contrast and specificity [34]. In comparison to conventional brightfield microscopy that is based on transmitted light, fluorescence microscopy selectively illuminates and images fluorescently labeled structures, rendering it an indispensable tool in cell biology, molecular biology, and virology [41].

The basic principle of fluorescence microscopy relies on fluorophore excitation and emission. A fluorophore, upon absorbing light at a particular wavelength, gets excited and, in doing so, transitions to the ground state by releasing light at a longer wavelength [25]. The fluorescence emitted is captured with the help of optical filters, and this enables scientists to view structures with decent spatial resolution. Specificity for fluorescence microscopy is obtained by labeling fluorophores to antibodies, proteins, or nucleic acids, enabling the examination of cellular structures with great precision [41].

Types of Fluorescence Microscopy

Several fluorescence microscopy techniques have been designed to enhance resolution, contrast, and specificity. The most common ones are:

1. Widefield Fluorescence Microscopy: The most common and simplest type of fluorescence microscopy, where the whole sample is illuminated with a wide light source. This refinement significantly enhances optical sectioning and produces sharper, more detailed images—especially useful for analysing thicker samples. The technique improves resolution and contrast by point-by-point scanning in the sample, rendering crisp three-dimensional images. It is especially suitable for thick samples and live-cell imaging [42].

2. Confocal Laser Scanning Microscopy (CLSM) overcomes a critical deficiency present in widefield fluorescence, namely the effect of out-of-focus light masking the quality of the image. By the addition of a very small pinhole designed to eliminate the unwanted light from the system, as well as the scanning of the specimen point by point, CLSM yields images with greatly enhanced resolution and contrast [49]. The technique thus proves particularly useful in the examination of thicker biological samples or the examination of living cells over time in real time [53].

3. Super-Resolution Microscopy: The diffraction limit, once a limitation on traditional fluorescence microscopy, restricts resolution to about 200 nm. Yet, super-resolution techniques such as Stimulated Emission Depletion (STED), Stochastic Optical Reconstruction Microscopy (STORM), and Photoactivated Localization Microscopy (PALM) have broken this barrier, allowing the imaging of structures at resolutions of less than 50 nm [30].

Live-Cell Imaging

Live-cell imaging is a sophisticated microscopy method that aims to study dynamic biological processes in living cells over a period. In contrast to fixed-cell imaging, which takes static images, live-cell imaging enables researchers to monitor intracellular processes like protein trafficking, mitosis, autophagy, viral infection, and immune cell function in real time [11]. Live-cell imaging has emerged as a key strategy for observing intricate biological processes in situ, as they occur in the cell's own internal environment. Using advanced microscopy techniques, researchers are now capable of acquiring high-quality images of cells in real-time, which enable them to see structure and function changing as it happens. What is distinct about this method is that it retains the native cell state — by not using chemical fixation or staining, it effectively reduces interference with normal cell function to a bare minimum [4].

This has made live-cell imaging a critical methodology in virology, neuroscience, cancer biology, and

drug discovery. One of the minimum requirements for effective live-cell imaging is keeping cell viability in the microscope. This includes rigorous temperature, pH, and osmotic balance control, typically provided by specialized imaging chambers. Imaging methods must also keep phototoxicity to a minimum since living cells can be harmed and their behaviors changed by being exposed to light for extended periods [56].

Techniques in Live-Cell Imaging

Fluorescence live-cell imaging is among the most popular methods used for observing the beautiful ballet of cell dynamics. This technique relies either on genetically encoded fluorescent proteins, like GFP and RFP, or on chemical dyes with specific affinity for particular cellular structures. Despite the exceptional specificity and contrast of these fluorescence-based techniques, they are susceptible to photobleaching, a phenomenon in which ongoing light exposure deteriorates fluorescence intensity over time [52].

One of the other widely utilized techniques is Total Internal Reflection Fluorescence (TIRF) Microscopy, which is a technique for selective imaging of molecules close to the plasma membrane. TIRF produces an evanescent wave that excites fluorophores in only a thin layer, roughly 100 nanometers, close to the coverslip. This unique characteristic makes it very suitable for examining membrane-associated processes such as receptor-ligand interactions, endocytosis, and viral entry [55].

Two-photon microscopy offers greater tissue penetration and reduced phototoxicity compared to confocal microscopy. Near-infrared light is used to excite fluorophores at deeper levels in this method and hence it is well suited for imaging live tissues and organisms [49]. Two-photon microscopy has been instrumental in neuroscience studies, especially in monitoring synaptic activity and interactions of neural networks.

The other techniques involve Fluorescence Recovery After Photobleaching (FRAP), a technique that gauges the mobility and diffusion of proteins by photobleaching a region and watching the recovery of ensuing fluorescence. Another technique is Fluorescence Lifetime Imaging Microscopy (FLIM), which monitors the decay of fluorescence to disclose details about the biochemical environment of live cells [26].

Electron Microscopy (EM) Techniques

Electron microscopy (EM) is a sophisticated method that employs electron beams instead of light to obtain extremely high-resolution images of biological specimens. Due to the much smaller wavelength of electrons, the technique provides much better resolution than traditional light microscopy [67]. It allows the examination of subcellular structures, viruses, and large molecular aggregates at the nanometer resolution and, in some instances, even at the angstrom resolution. Electron microscopy has become especially important in structural biology and virology, where detailed fine structures need to be understood [22].

Transmission Electron Microscopy (TEM)

Transmission electron microscopy (TEM) is the most popular EM method of analyzing biological samples. TEM involves directing a beam of high-energy electrons through a specimen of 50–100 nm thickness that is made ultrathin. Contrast due to electron scattering resulting from electron interaction in the specimen produces high-image resolution of internal structures [58].

In virology, TEM was pivotal in the determination of viral structure, morphology, and intracellular associations. TEM observations in early days, for example, provided the initial direct observation of viruses like the poliovirus and the influenza virus, revealing their capsid symmetry and surface topography. TEM was also widely used to examine virus-induced changes in cells, e.g., replication organelles, virus-host associations [23].

One major benefit of TEM is the possibility to image structures at near atomic level resolution. Nevertheless, it involves complex sample preparation through fixation, dehydration, and embedding in resin, which may introduce artifacts. TEM is also generally performed on fixed samples and therefore is not appropriate for live-cell imaging.

Scanning Electron Microscopy (SEM)

In contrast to TEM, SEM provides high-resolution images of the topography of the biological samples' surfaces and not of internal structures. SEM operates by scanning the surface of the specimen using a beam of focused electrons and generating secondary and backscattered electrons in the creation of a three-dimensional image [29].

SEM is also particularly beneficial in studying virus morphology, viral entry mechanisms, and cell surface interactions [44]. SEM was used, for example, to study how coronavirus attaches to host cell receptors and induces membrane fusion. SEM was also used to study viral budding and cell egress of infected cells.

One of the significant advantages of SEM is that it is capable of generating high-quality 3D cell structure reconstructions. Yet, like TEM, SEM also requires complicated sample preparation involving chemical fixation of the samples, drying or dehydration, and coating them in conductive materials, which are gold or platinum, to prevent electron charging effects [71].

Cryo-Electron Microscopy (Cryo-EM)

Cryo-Electron Microscopy or Cryo-EM has led to a revolution in the study of structural biology by enabling the imaging of biological macromolecules in their natural state of hydration. Unlike TEM and SEM in which samples get processed exten-

sively, Cryo-EM quickly quenches samples in liquid ethane to immobilize the structure without chemical fixation [10].

Cryo-EM has also been the preferred approach for the determination of high-resolution viral protein structures, e.g., SARS-CoV-2 spike proteins, influenza hemagglutinin, and HIV-1 envelope glycoproteins. Single-particle imaging in the thousands and computer-assisted 3D structure reconstruction by Cryo-EM allow researchers to study the conformational dynamics of viral proteins and identify potential drug targets [40].

Cryo-EM provides several advantages over traditional EM methods, e.g., fewer preparations of samples, imaging of samples in their state of hydration, and the potential for near-atomic resolution. Specialized equipment is required, e.g., cryo-enabled microscopes and high-performance image processing computing equipment [10].

Cryo-Electron Tomography (Cryo-ET)

Cryo-Electron Tomography (Cryo-ET) is the technologically advanced extension of Cryo-EM for 3D imaging of cellular structures at the molecular resolution level. Unlike single-particle Cryo-EM, where 3D structures are reconstructed from average projections of extremely large particle numbers, Cryo-ET includes the step of tilting a single specimen at various angles and computationally reconstructing a 3D tomogram [27].

Cryo-ET has played a crucial role in investigating cell modifications induced by viruses, including replication compartments and virus-host interactions. Cryo-ET, for instance, gave detailed information on coronavirus replication compartments' structure and unveiled the viral RNA synthesis machine. Cryo-ET has also imaged viral entry processes, such as influenza virus particle membrane fusion [61].

One of the advantages of Cryo-ET is that it can potentially yield high-resolution 3D reconstructions of the full cell context. It is technically challenging, however, and needs expert sample preparation, high-precision imaging, and computationally intensive processing.

Correlative and Multiscale Imaging

Advances in imaging technologies have given rise to correlative and multiscale imaging methodologies that use various microscopy methods to image the molecular structures and interactions of cells in various resolutions and scales. These methodologies bridge the gap between light microscopy (LM) and electron microscopy (EM) and allow researchers to correlate dynamic live-cell imaging with high-resolution structural information. These methods include Correlative Light and Electron Microscopy (CLEM), FIB-SEM, SBF-SEM, and Helium-Ion Microscopy (HIM), which are potent methodologies to explore virus-host interactions, cell ultrastructure, and sub-cellular processes [2].

Correlative Light and Electron Microscopy (CLEM)

Correlative Light and Electron Microscopy combines the dynamic live-cell imaging capabilities of fluorescence microscopy and the high resolution of electron microscopy. It is particularly well adapted for tracking of viral infection, protein localization, and ultrastructure of cells since it allows researchers to correlate fluorescent-labeled molecules and ultrastructure in the same specimen [59].

In virology, CLEM has also come to be used in virus-host interactions by initially imaging live cells by fluorescent microscopy to trace viral entry, replication, and egress. These samples are subsequently prepared for electron microscopy to capture ultrastructure of viral particles and host cell organelles [39]. For example, CLEM has traditionally come to be used in studying SARS-CoV-2 replication compartments and how viral RNA synthesis sites correlate to host cell organelles.

A significant strength of CLEM is that it allows both dynamic, live-cell imaging and high-resolution structural data to be combined. It is, however, subject to rigorous light and electron microscopy image registration and hence is technically demanding [62].

Volume Electron Microscopy (FIB-SEM, SBF-SEM)

Volume Electron Microscopies (vEM) are methods by which 3D reconstruction of biological specimens can be made in nanometer resolution. Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) and Serial Block-Face Scanning Electron Microscopy (SBF-SEM) are the two popular vEM technologies. Scientists use these technologies to study the architecture of the spatial arrangement of subcellular components and large biological volumes, which is beneficial in cell biology and virology [37].

FIB-SEM utilizes a focused ion beam serially to mill away samples in thin layers in combination with SEM imaging of the exposed surface. It produces a high-definition 3D structure data set of cells. FIB-SEM was used to study viral replication compartments, mitochondrial reorganization, and viral inclusions in the cytoplasm [68].

SBF-SEM employs a diamond knife to slice the sample in very thin sections for SEM imaging. It is particularly beneficial in the examination of large tissue samples, organoids, and whole infected cells since it allows cell changes caused by the virus to be examined in whole cell populations.

Volume Electron Microscopy is also extremely useful in virus-host interaction research because of the ultrastructural context it provides for viral replication and assembly sites. Its drawback is that it is labor-intensive, equipment-dependent, and generates incredibly large data sets that require high-level computational analysis [37].

Helium-Ion Microscopy (HIM)

Helium-Ion Microscopy (HIM) is one of the new sub-nanometer resolution imaging technologies that

performs high-resolution imaging of biological samples using very minimal sample preparation. While traditional SEM employs an electron beam, HIM utilizes a helium ion beam that possesses numerous advantages like increased surface sensitivity, decreased damage to the samples, and improved contrast for biological samples [51].

HIM is particularly well-suited to image biological membranes, viruses, and cell surfaces. It has also been used to study viral particle surface topology, host cell entry, and structures of biofilms. Its ability to image without conductive coatings makes it well-suited to image sensitive biological specimens [65].

However, HIM is still a relatively new technology, and application to biological sciences is constrained by cost and requirement for specialized know-how. In spite of these impediments, HIM holds the potential to revolutionize nanoscale imaging of cell structures and viruses [18].

Molecular and Functional Imaging

Molecular and functional imaging technologies are strong methodologies for the study of biomolecule localization, interactions, and functional activities in cells. They find special application in cell biology and virology, where it is possible to visualize the high-resolution spatial distribution of viral and host protein, nucleic acid, and metabolism. Immunogold Labeling, In Situ Hybridization (ISH), and EM Autoradiography are among the most frequently used methods, which utilize a variety of molecular tagging and detection methods in dissecting the structure and function of biological entities in infection and disease.

Immunogold Labeling

Immunogold labeling refers to a high-resolution electron microscopy (EM) technique applied in protein or biomolecule localisation in tissue and cells. It utilizes gold nanoparticles tagged to antibodies that selectively attach to the molecules of interest. Gold particles happen to be electron-dense and therefore under Transmission Electron Microscopy (TEM) or Scanning Electron Microscopy (SEM) provide high-contrast black spots, enabling protein localisation to the nanometer level [16].

In virology, immunogold labeling enabled the distribution of viral protein in infected cells to be mapped. For example, the method was used in investigating the localisation of the SARS-CoV-2 nucleocapsid protein in replication compartments, providing knowledge of how it interacts with host cell membranes [6]. It also enabled determination of protein-protein interactions in the ultrastructure, e.g., of viral polymerases with host cell ribosomes. However, one of the disadvantages of immunogold labeling is that the fixation and embedding of the samples may result in a loss of antigenicity, hence less efficient labeling. Dual or multi-labeling to examine interactions between several proteins also requires optimization of sizes of gold particles and of antibody concentrations [69].

In Situ Hybridization (ISH)

In Situ Hybridization (ISH) is a powerful molecular imaging tool that is used for the identification and localization of specific nucleic acid sequences (RNA or DNA) in cells and tissue. It allows for the study of gene expression, viral genome location, and genetic alteration through the hybridization of a labeled probe to complementary nucleic acid sequences [20].

ISH also finds extensive use in virology in the detection of viral RNA and DNA in virus-infected cells. For example, Fluorescence In Situ Hybridization (FISH) was applied in the study of HIV-1 RNA host nuclei distribution, where crucial replication sites of the virus were made known. FISH was also applied to examine host gene expression changes following viral infection, where cell defense mechanism information was made available [24]. Oncology also greatly benefits from ISH, where the method is applied in the detection of chromosomal aberrations and oncogene amplification in cancer.

Although ISH is specific and sensitive, it also has some disadvantages. Cross-hybridizations of probes may lead to false positives, and the method is also tedious. ISH also requires proper RNA or DNA preservation, and therefore, preparation of the samples is of extreme significance in obtaining reproducible results [14].

EM Autoradiography

Electron Microscopy (EM) Autoradiography is a cutting-edge molecular imaging method used in tracing cellular metabolism and molecular dynamics. It identifies the occurrence of radioactively labeled molecules on high-resolution EM imaging.

This technique is of valuable application in following viral replication with pulse-labeling at infection with radiolabeled nucleotides. EM autoradiography, for instance, was used in demonstration of influenza virus RNA synthetic sites by following the destiny of radiolabeled RNA precursors into nascent viral genomes. This technique is also used in protein and lipid metabolic labeling where researchers follow protein synthesis locations, lipid metabolism, and virus assembly places [48].

A significant advantage of EM autoradiography is that it can follow the dynamics of molecules at high spatial resolution and is thus highly appropriate to examine the intracellular trafficking and replication of viral genomes. The technique is, however, outweighed by several disadvantages in the form of long exposure times, demands of radioactive facilities, and time-consuming image analysis.

Applications of Imaging Techniques in Virus-Cell Interactions

Imaging modalities have revolutionized the way that scientists investigate virus-host interactions since now for the first time ever it is possible to visualize directly the critical viral processes of host cell

entry, intracellular traffic, replication, assembly, and host cell structural remodeling. Improvements in and optimization of advanced microscopy techniques—particularly fluorescence microscopy, live-cell imaging, electron microscopy, and correlative integrated imaging—have introduced unprecedented new levels of investigation in the study of viral infections. These techniques have enabled scientists to watch viral activity in real time, trace their path through the host cell, and examine the fine structural organization of replication sites with a precision never before attainable [33].

Studying Viral Entry and Trafficking

Imaging of interaction of viruses with the host cell receptors, i.e., for HIV-1, Influenza, and SARS-CoV-2, has made it more evident as for viral entry itself, which constitutes the initial infection stage. Throughout this phase, viruses attach to specific receptors of the host cell surface, prompting subsequent signaling inducing membrane fusion or endocytic uptake. Advanced imaging technologies, particularly single-molecule Förster Resonance Energy Transfer (smFRET), have been crucial for recording conformational changes of viral spike proteins upon binding with host receptors and progression toward membrane fusion [1]. SARS-CoV-2, HIV-1, and influenza virus have been investigated to reveal that their spike proteins quickly change their structure on binding with host receptors ACE2 (SARS-CoV-2), CD4 (HIV-1), and sialic acid residues (Influenza) [66].

In addition to smFRET, live-cell fluorescence microscopy has also been crucial in the tracking of real-time virus particle motion on the cell surface. Total Internal Reflection Fluorescence Microscopy (TIRF) has been particularly helpful in imaging on or near the plasma membrane and in providing high-resolution views of viral particle attachment to host receptors and into entry. Apart from that, development of super-resolution microscopy methods like Stochastic Optical Reconstruction Microscopy (STORM) and Photoactivated Localization Microscopy (PALM) made the visualization possible of individual isolated viral particles to a nanometer resolution, and so the viral receptors' cell surface spatial organization and clustering pattern are revealed [47].

Viral genomes delivery to the sites of replication in the cytoplasm constitutes a crucial event of the infecting process when the entry into cells is efficient. Imaging techniques involving fluorescently labeled viral nucleic acids enabled researchers to monitor intracellular dynamics of these genomes in real time. Live-cell microscopy, for instance, has demonstrated how HIV-1 reverse transcription complexes are transported actively along microtubule tracks to the nucleus, a process mediated by dynein motor protein. Building on these findings, electron microscopy has allowed imaging of virus-containing vesicles and their targeted delivery to specific subcellular compartments, resolving further our understanding of intracellular viral transport [2].

Visualization of Viral Replication and Assembly

Electron tomography of coronavirus, enterovirus, and flavivirus replication factories. Upon entry into the cell, viruses reorganize host cell membranes to create replication organelles (ROs), which serve as platforms for viral genome replication. Multiscale electron microscopy techniques, such as electron tomography and volume scanning electron microscopy (SEM), have provided 3D reconstructions of such replication structures. Studies of coronaviruses (SARS-CoV-2, MERS-CoV), enteroviruses (coxsackievirus, polio), and flaviviruses (Dengue, Zika) have revealed complex networks of membranes facilitating viral genome formation [67]. For instance, electron tomography demonstrated that SARS-CoV-2 induces endoplasmic reticulum-derived double-membrane vesicles (DMVs), which are special sites for viral RNA replication. These are likely protected from host immune sensors, thus allowing for efficient replication while evading detection [64].

Live-cell microscopy for viral protein localization tracking. Live-cell imaging has been important in elucidating the spatiotemporal dynamics of viral protein localization in infection. Fluorescently labeled viral proteins allow researchers to monitor their motion and interactions with host factors in real time [56]. For example, fluorescently tagged Ebola virus matrix proteins have been employed to illustrate how these proteins accumulate at the plasma membrane before virus budding. Likewise, GFP-labeled influenza nucleoproteins have been followed to discern their nuclear import during replication.

Host Cell Remodeling During Infection

Viral replication organelles (ROs) and their effects on cell structure. The majority of viruses induce extreme modifications of host cell structure to facilitate proper conditions for replication. Imaging studies have shown that positive-strand RNA viruses such as coronaviruses and flaviviruses form complex membrane-bound structures in which viral replication occurs. Cryo-electron tomography (Cryo-ET) has provided high-resolution data on how such viral ROs assemble and are organized, including the illustration of specialized channels that permit RNA export but protect viral genomes from host immunity [27].

Besides membrane restructuring, certain viruses even hijack host cytoskeletal components. For example, live-cell imaging studies of Herpes Simplex Virus (HSV-1) have shown the virus reorganizes the networks of actin and microtubules actively to facilitate intracellular trafficking and egress.

3D visualization of virus-induced changes in the membrane. FIB-SEM and SBF-SEM are ultra-high-resolution imaging techniques that have been used

to visualize 3D virus-induced changes in membranes. These technologies have revealed that Dengue virus-infected cells develop invaginated spherules, which serve as replicative sites as well as aid viral genome synthesis in a safe environment [66].

Challenges and Limitations in Virus Imaging

Despite dramatic advances in imaging techniques, there are several challenges and limitations in virus imaging. These arise due to the resolution-sample preparation trade-off, problems with labeling and contrast enhancement, limitations in capturing dynamic virus-host interactions, and biosafety concerns. Enhancing the accuracy and efficiency of virus imaging, particularly for highly pathogenic viruses such as SARS-CoV-2, Ebola, and HIV-1, therefore relies on addressing these issues [33].

Resolution vs Sample Preparation Trade-offs

The one largest limitation to virus imaging is the resolution-vs-sophisticic sample preparation trade-off. While, with high resolutions such as Cryo-Electron Microscopy (Cryo-EM) and Electron Tomography (Cryo-ET), imaging modes provide near atomic-level resolutions, they also require a sophisticated level of sample preparation [3].

Complex sample processing is required for high-resolution Electron Microscopy (EM). Standard Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) involve complicated sample preparation, i.e., chemical fixation, dehydration, staining, resin embedding, and ultrathin sectioning. These steps can cause artifacts that lead to misinterpretation of viral structures.

Cryo-EM is low-throughput and expensive. Cryo-EM has transformed structural virology with the facility to image native hydrated viruses without chemical fixation. However, Cryo-EM requires expensive equipment, specialized expertise, and is low-throughput and, therefore, not readily adoptable by the majority of research labs. Cryo-ET datasets are also large and computationally demanding and require advanced image processing software for 3D structure reconstruction.

The need for enhanced sample preparation techniques which preserve ultrastructure and are time-saving is crucial in order to improve virus imaging efficiency [44].

Limitations in Labeling and Contrast Enhancement

Labeling and contrast enhancement are crucial for visualizing viral components, but existing techniques have several limitations. Electron Microscopy (EM) lacks natural contrast for biological samples, requiring the use of heavy metal stains, while fluorescence-based imaging is limited by photobleaching and labeling specificity.

Immunolabeling challenges in EM. Immunogold labeling is widely used to detect viral proteins, but it suffers from low labeling efficiency, steric hindrance, and antigen loss during sample preparation. Furthermore, multiple labeling for co-localization studies is difficult, as different-sized gold particles must be used to distinguish between various proteins.

Need for new probes and staining methods. In fluorescence microscopy, genetically encoded fluorescent proteins (e.g., GFP, RFP) provide high specificity but may alter protein function. Chemical dyes can be non-specific, leading to background fluorescence. Developing novel, brighter, and more photostable probes is essential for improving virus imaging.

Future advancements in super-resolution microscopy and correlative imaging techniques may help overcome these labeling limitations by enabling higher specificity and contrast in virus imaging [37].

Difficulties in Studying Dynamic Virus-Host Interactions

Virus-host interactions to be replicated in real time remain a significant issue, mainly due to the incredible speeds at which viral processes are carried out and the constraints at the moment on available imaging technologies. Processes like membrane fusion, emission of the viral genome, and RNA synthesis are carried out in a millisecond to a few seconds and are thus events stupendously difficult to image through conventional microscopy. These transient dynamics not only need sophisticated imaging devices with ultra-high temporal resolution but also innovative approaches to preserve biological relevance with visualization.

Imaging of fast processes like viral fusion and RNA replication. Live-cell fluorescence microscopy single-virus tracking approaches have provided insights into viral entry and traffic. They are hindered by limited temporal resolution, photobleaching, and phototoxicity. Microscopic strategies like smFRET (single-molecule FRET) and TIRF (Total Internal Reflection Fluorescence Microscopy) improved the resolution but still have a hard time to image sub-second events.

Blending live-cell imaging and high-resolution EM remains a challenge. Correlative Light and Electron Microscopy (CLEM) has emerged as a promising approach for correlating live-cell fluorescence imaging and ultrastructural EM, yet it remains technically challenging and time-consuming.

To overcome these challenges, AI-assisted image analysis and high-speed super-resolution imaging can hopefully create a more precise picture of viral dynamics in the future [41].

Biosafety Concerns and Access to Advanced Imaging Facilities

Handling highly pathogenic viruses requires strict biosafety protocols, and imaging availability is severely limited thereby. High-level biosafety laboratories (BSL-3 and BSL-4) are needed for handling

viruses like SARS-CoV-2, Ebola, and Marburg virus, making the experiment difficulty and resource demand higher.

Working with highly pathogenic viruses (e.g., SARS-CoV-2, Ebola). Cryo-EM, live-cell imaging, and super-resolution microscopy require special containment measures to prevent exposure. Fixation and inactivation methods must be optimized to preserve viral structure while ensuring biosafety.

Requirement of high-containment biosafety facilities for imaging. Imaging infectious viruses in a living form require access to BSL-3 or BSL-4 level facilities, which are available only at centers of special expertise. Some imaging techniques such as cryo-EM and FIB-SEM also require expensive instrumentation available only at premier institutes.

To overcome these limitations, other virus models such as pseudoviruses and virus-like particles (VLPs) are underway to utilize for imaging studies under lower biosafety conditions. Certain initiatives towards developing more accessible imaging core facilities also help in democratising the access of advanced virus imaging technologies [5].

Conclusion

Imaging techniques have transformed virus–cell interaction research, enabling scientists to track viral entry, replication, assembly, and host response with unprecedented resolution [67]. Light microscopy, including fluorescence and super-resolution microscopy, has provided real-time viral dynamic information, whereas electron microscopy (EM) methods have yielded high-resolution structural information on viral particles and replication sites. The correlative application of imaging modalities has also bridged the gap between molecular and ultrastructural virology and has yielded a composite picture of viral infection [40].

Despite these advances, there are still some challenges, for instance, in achieving an optimum resolution-sample preparation trade-off, enhancing labeling efficiency, and capturing fast virus-host interactions. Biosafety level high-containment requirements for imaging highly pathogenic viruses also impose accessibility restrictions. However, ongoing development of AI-driven image analysis, nanotechnology-based imaging probes, and high-speed live-cell imaging will bypass many of these restrictions [52].

Moving ahead, the future of viral imaging lies in integrating multiple imaging modalities with computational and molecular approaches to obtain an even more comprehensive and dynamic view of viral infection [34]. By continuing to advance imaging approaches and overcome current challenges, scientists will certainly continue to clarify important details of viral pathogenesis, which will in turn aid in the development of effective antiviral therapies, vaccines, and diagnostics.

References

1. Bernhard O.K., Diefenbach R.J., Cunningham A.L. New insights into viral structure and virus–cell interactions through proteomics. *Expert Rev. Proteomics, 2005, vol. 2, no. 4, pp. 577–588. doi: 10.1586/14789450.2.4.577*
2. Bykov Y.S., Cortese M., Briggs J.A.G., Bartenschlager R. Correlative light and electron microscopy methods for the study of virus–cell interactions. *FEBS Lett., 2016, vol. 590, no. 13, pp. 1877–1895. doi: 10.1002/1873-3468.12153*
3. Chen T., Tu S., Ding L., Jin M., Chen H., Zhou H. The role of autophagy in viral infections. *J. Biomed. Sci., 2023, vol. 30, no. 1: 5. doi: 10.1186/s12929-023-00899-2*
4. Cole R. Live-cell imaging. *Cell Adh. Migr., 2014, vol. 8, no. 5, pp. 452–459. doi: 10.4161/cam.28348*
5. Cornish N.E., Anderson N.L., Arambula D.G., Arduino M.J., Bryan A., Burton N.C., Cohn A.C., Dallas S.D., Gerber S.I., Hayden R.T., Huang J., Jerris R.C., Kocagöz S., Kocagöz T., Kuhar D.T., Larone D.H., Mahoney M.V., Perkins K.M., Polage C.R., Raney K.D., Richter S.S., Salfinger M., Schlaberg R., Török T.J., Wolk D.M., Yarita K., Ye X. Clinical Laboratory Biosafety Gaps: Lessons Learned from Past Outbreaks Reveal a Path to a Safer Future. *Clin. Microbiol. Rev., 2021, vol. 34, no. 3: e0012618. doi: 10.1128/CMR.00126-18*
6. Cuervo A.M., Knecht E., Terlecky S.R., Dice J.F. Activation of a selective pathway of lysosomal proteolysis in rat liver by prolonged starvation. *Am. J. Physiol., 1995, vol. 269, no. 5, Pt 1: C1200. doi: 10.1152/ajpcell.1995.269.5.c1200*
7. DiGiuseppe S., Bienkowska-Haba M., Sapp M. Human Papillomavirus Entry: Hiding in a Bubble. *J. Virol., 2016, vol. 90, no. 18, pp. 8032–8035. doi: 10.1128/JVI.01065-16*
8. Dimitrov D.S. Virus entry: molecular mechanisms and biomedical applications. *Nat. Rev. Microbiol., 2004, vol. 2, no. 2, pp. 109–122. doi: 10.1038/nrmicro817*
9. Dobbie I.M. Bridging the resolution gap: correlative super-resolution imaging. *Nat. Rev. Microbiol., 2019, vol. 17, no. 6: 337. doi: 10.1038/s41579-019-0207-6*
10. Earl L.A., Falconieri V., Milne J.L., Subramaniam S. Cryo-EM: Beyond the microscope. *Curr. Opin. Struct. Biol., 2017, vol. 46, pp. 71–78. doi: 10.1016/j.sbi.2017.06.002*
11. Ettinger A., Wittmann T. Fluorescence live cell imaging. *Methods Cell Biol., 2014, vol. 123, pp. 77–94. doi: 10.1016/B978-0-12-420138-5.00005-7*
12. Fish K.N. Total internal reflection fluorescence (TIRF) microscopy. *Curr. Protoc. Cytom., 2009, vol. 50, no. 1, pp. 12.18.1–12.18.13. doi: 10.1002/0471142956.cy1218s50*
13. Giacomelli G. Spatiotemporal localization of proteins in microorganisms via photoactivated localization microscopy. *2021, vol. 4, no. 6, pp. 2–13. doi: 10.5282/edoc.27360*
14. Haase A., Brahic M., Stowring L., Blum H. Detection of viral nucleic acids by in situ hybridization. *In: Methods in Virology. Vol. 7. New York: Academic Press, 1984, pp. 189–226. doi: 10.1016/B9780124702073.500139*

15. Hell S.W., Wichmann J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt. Lett., 1994, vol. 19, no. 11: 780. doi: 10.1364/OL.19.000780*
16. Hermann R., Walther P., Müller M. Immunogold labeling in scanning electron microscopy. *Histochem. Cell Biol., 1996, vol. 106, no. 1, pp. 31–39. doi: 10.1007/BF02473200*
17. Hess S.T., Girirajan T.P.K., Mason M.D. Ultra-high resolution imaging by fluorescence photoactivation localization microscopy. *Biophys. J., 2006, vol. 91, no. 11, pp. 4258–4272. doi: 10.1529/biophysj.106.091116*
18. Hlawacek G., Veligura V., van Gastel R., Poelsema B. Helium ion microscopy. *J. Vac. Sci. Technol. B, 2014, vol. 32, no. 2. doi: 10.1116/1.4863676*
19. Hoenen T., Groseth A. Virus–Host Cell Interactions. *Cells, 2022, vol. 11, no. 5: 804. doi: 10.3390/cells11050804*
20. Jensen E., Crossman D.J. Technical review: Types of imaging — Direct STORM. *Anat. Rec., 2014, vol. 297, no. 12, pp. 2227–2231. doi: 10.1002/ar.22960*
21. Johnson D.S., Jaiswal J.K., Simon S. Total internal reflection fluorescence (TIRF) microscopy illuminator for improved imaging of cell surface events. *Curr. Protoc. Cytom., 2012, vol. 61, no. 1, pp. 12.29.1–12.29.19. doi: 10.1002/0471142956.cy1229s61*
22. Junod S.L., Saredy J., Yang W. Nuclear import of adeno-associated viruses imaged by high-speed single-molecule microscopy. *Viruses, 2021, vol. 13, no. 2: 167. doi: 10.3390/v13020167*
23. Laue M. Electron Microscopy of Viruses. *Methods Cell Biol., 2010, vol. 96, pp. 1–20. doi: 10.1016/S0091-679X(10)96001-9*
24. Levsky J.M., Singer R.H. Fluorescence in situ hybridization: past, present and future. *J. Cell Sci., 2003, vol. 116, no. 14, pp. 2833–2838. doi: 10.1242/jcs.00633*
25. Lichtman J.W., Conchello J.A. Fluorescence microscopy. *Nat. Methods, 2005, vol. 2, no. 12, pp. 910–919. doi: 10.1038/nmeth817*
26. Lu M. Single-molecule FRET imaging of virus spike–host interactions. *Viruses, 2021, vol. 13, no. 2: 332. doi: 10.3390/v13020332*
27. Lucic V., Leis A., Baumeister W. Cryo-electron tomography of cells: Connecting structure and function. *Histochem. Cell Biol., 2008, vol. 130, no. 2, pp. 185–196. doi: 10.1007/s00418-008-0459-y*
28. McClelland R.D., Culp T.N., Marchant D.J. Imaging Flow Cytometry and Confocal Immunofluorescence Microscopy of Virus-Host Cell Interactions. *Front. Cell. Infect. Microbiol., 2021, vol. 11: 749039. doi: 10.3389/fcimb.2021.749039*
29. Mohammed A., Abdullah A. Scanning electron microscopy (SEM): A review. *Proceedings of 2018 International Conference on Hydraulics and Pneumatics — HERVEX. November 7–9, Băile Govora, Romania, pp. 77–85.*
30. Mukherjee S., Boutant E., Réal E., Mély Y., Anton H. Imaging viral infection by fluorescence microscopy: Focus on HIV-1 early stage. *Viruses, 2021, vol. 13, no. 2: 213. doi: 10.3390/v13020213*
31. Murphy D.B. Digital light microscopy techniques for the study. In: Murphy D.B., editor. Fundamentals of Light Microscopy and Electronic Imaging. *New York: Wiley-Liss, 1999, pp. 1–32*
32. Müller T., Schumann C., Kraegeloh A. STED Microscopy and its Ap plications: New Insights into Cellular Processes on the Nanoscale. *ChemPhysChem, 2012, vol. 13, no. 8, pp. 1986–2000. doi: 10.1002/cphc.201100986*
33. Müller T.G., Sakin V., Müller B. A Spotlight on Viruses — Application of Click Chemistry to Visualize Virus-Cell Interactions. *Molecules, 2019, vol. 24, no. 3: 481. doi: 10.3390/molecules24030481*
34. Nickerson A., Huang T., Lin L.J., Nan X. Photoactivated localization microscopy with bimolecular fluorescence complementation (BiFC-PALM). *J. Vis. Exp., 2015, vol. 106: e53154. doi: 10.3791/53154*
35. Parveen N., Borrenberghs D., Rocha S., Hendrix J. Single viruses on the fluorescence microscope: Imaging molecular mobility, interactions and structure sheds new light on viral replication. *Viruses, 2018, vol. 10, no. 5: 250. doi: 10.3390/v10050250*
36. Payne S. Virus Interactions With the Cell. *Viruses, 2017, pp. 23–35. doi: 10.1016/B978-0-12-803109-4.00003-9*
37. Peddie C.J., Genoud C., Kreshuk A., Meechan K., Micheva K.D., Narayan K., Pape C., Parton R.G., Polishchuk R.S., Ronchi P., Schieber N.L., Schwab Y., Steyer A.M., Swedlow J.R., Verkade P., Briggs J.A.G. Volume electron microscopy. *Nat. Rev. Methods Primers, 2022, vol. 2: 51. doi: 10.1038/s43586-022-00131-9*
38. Rajcani J. Molecular mechanisms of virus spread and virion components as tools of virulence: A review. *Acta Microbiol. Immunol. Hung., 2003, vol. 50, no. 4, pp. 407–431. doi: 10.1556/AMicr.50.2003.4.8*
39. Razi M., Tooze S.A. Chapter 17 Correlative light and electron microscopy. *Methods Enzymol., 2009, vol. 452, pp. 261–275. doi: 10.1016/S0076-6879(08)03617-3*
40. Richert-Pöggeler K.R., Franzke K., Hipp K., Kleespies R.G. Electron microscopy methods for virus diagnosis and high resolution analysis of viruses. *Front. Microbiol., 2019, vol. 10: 421852. doi: 10.3389/fmicb.2018.03255*
41. Risco C. Application of Advanced Imaging to the Study of Virus-Host Interactions. *Viruses, 2021, vol. 13, no. 10: 1958. doi: 10.3390/v13101958*
42. Robb N.C. Virus morphology: Insights from super-resolution fluorescence microscopy. *Biochim. Biophys. Acta Mol. Basis Dis., 2022, vol. 1868, no. 4: 166347. doi: 10.1016/j.bbadis.2022.166347*
43. Rust M.J., Bates M., Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nat. Methods, 2006, vol. 3, no. 10, pp. 793–795. doi: 10.1038/nmeth929*
44. Ryan J., Gerhold A.R., Boudreau V., Smith L., Maddox P.S. Introduction to modern methods in light microscopy. *Methods Mol. Biol., 2017, vol. 1563, pp. 1–15. doi: 10.1007/978-1-4939-6810-7_1*
45. Saffarian S. Application of advanced light microscopy to the study of HIV and its interactions with the host. *Viruses, 2021, vol. 13, no. 2: 223. doi: 10.3390/v13020223*
46. Saibil H., White N. Recent advances in biological imaging. *Biosci. Rep., 1989, vol. 9, no. 4, pp. 437–449. doi: 10.1007/BF01117046*
47. Sakin V., Paci G., Lemke E.A., Müller B. Labeling of virus components for advanced, quantitative imaging analyses. *FEBS Lett., 2016, vol. 590, no. 13, pp. 1896–1914. doi: 10.1002/1873-3468.12131*
48. Salpeter M.M., McHenry F.A. Electron microscope autoradiography. *Adv. Tech. Biol. Electron Microsc., 1973, pp. 113–152. doi: 10.1002/1873-3468.12131*
49. Sanderson M.J., Smith I., Parker I., Bootman M.D. Fluorescence microscopy. *Cold Spring Harb. Protoc., 2014, vol. 2014, no. 10: 071-795. doi: 10.1101/pdb.top071795*

50. Santarella-Mellwig R., Franke J., Jaedicke A., Gorjanacz M., Bauer U., Budd A., Mattaj I.W., Devos D.P. Correlative Light Electron Microscopy (CLEM) for Tracking and Imaging Viral Protein Associated Structures in Cryo-immobilized Cells. *J. Vis. Exp., 2018, vol. 139: e58154. doi: 10.3791/58154*
51. Schmidt M., Byrne J.M., Maasilta I.J. Bio-imaging with the helium-ion microscope: A review. *Beilstein J. Nanotechnol., 2021, vol. 12, pp. 1–23. doi: 10.3762/bjnano.12.1*
52. Schnell U., Dijk F., Sjollema K.A., Giepmans B.N.G. Immunolabeling artifacts and the need for live-cell imaging. *Nat. Methods, 2012, vol. 9, no. 2, pp. 152–158. doi: 10.1038/nmeth.1855*
53. Shotton D.M. Video-enhanced light microscopy and its applications in cell biology. *J. Cell Sci., 1988, vol. 89, no. Pt 2, pp. 129–150. doi: 10.1242/jcs.89.2.129*
54. Stewart P.L., Dermody T.S., Nemerow G.R. Structural basis of nonenveloped virus cell entry. *Adv. Protein Chem., 2003, vol. 64, pp. 455–491. doi: 10.1016/S0065-3233(03)01013-1*
55. Sun E., He J., Zhuang X. Live cell imaging of viral entry. *Curr. Opin. Virol., 2013, vol. 3, no. 1, pp. 34–43. doi: 10.1016/j.coviro.2013.01.005*
56. Sung M.H., McNally J.G. Live cell imaging and systems biology. *Wiley Interdiscip. Rev. Syst. Biol. Med., 2011, vol. 3, no. 2, pp. 167–182. doi: 10.1002/wsbm.108*
57. Tam J., Merino D. Stochastic optical reconstruction microscopy (STORM) in comparison with stimulated emission depletion (STED) and other imaging methods. *J. Neurochem., 2015, vol. 135, no. 4, pp. 643–658. doi: 10.1111/jnc.13257*
58. Tang C.Y., Yang Z. Transmission electron microscopy (TEM). *In: Membrane Characterization, 2017, pp. 145–159. doi: 10.1016/B978-0-444-63776-5.00008-5*
59. Timmermans F.J., Otto C. Contributed review: Review of integrated correlative light and electron microscopy. *Rev. Sci. Instrum., 2015, vol. 86, no. 1. doi: 10.1063/1.4905434*
60. Trache A., Meininger G.A. Total internal reflection fluorescence (TIRF) microscopy. *Curr. Protoc. Microbiol., 2008, vol. 10, no. 1, pp. 2A.2.1-2A.2.22. doi: 10.1002/9780471729259.mc02a02s10*
61. Turk M., Baumeister W. The promise and the challenges of cryo-electron tomography. *FEBS Lett., 2020, vol. 594, no. 20, pp. 3243–3261. doi: 10.1002/1873-3468.13948*
62. Van den Dries K., Fransen J., Cambi A. Fluorescence CLEM in biology: historic developments and current super-resolution applications. *FEBS Lett., 2022, vol. 596, no. 19, pp. 2486–2496. doi: 10.1002/1873-3468.14421*
63. Vicidomini G., Bianchini P., Diaspro A. STED super-resolved microscopy. *Nat. Methods, 2018, vol. 15, no. 3, pp. 173–182. doi: 10.1038/nmeth.4593*
64. Wang I.H., Burckhardt C.J., Yakimovich A., Greber U.F. Imaging, Tracking and Computational Analyses of Virus Entry and Egress with the Cytoskeleton. *Viruses, 2018, vol. 10, no. 4: 166. doi: 10.3390/v10040166*
65. Wirtz T., De Castro O., Audinot J.N., Philipp P. Imaging and analytics on the helium ion microscope. *Annu. Rev. Anal. Chem., 2019, vol. 12, pp. 523–543. doi: 10.1146/annurev-anchem-061318-115457*
66. Witte R., Andriasyan V., Georgi F., Yakimovich A., Greber U.F. Concepts in Light Microscopy of Viruses. *Viruses, 2018, vol. 10, no. 4: 202. doi: 10.3390/v10040202*
67. Wolff G., Bárcena M. Multiscale electron microscopy for the study of viral replication organelles. *Viruses, 2021, vol. 13, no. 2: 197. doi: 10.3390/v13020197*
68. Xu C.S., Hayworth K.J., Lu Z., Grob P., Hassan A.M., García-Cerdán J.G., Niyogi K.K., Nogales E., Weinberg R.J., Hess H.F. Enhanced FIB-SEM systems for large-volume 3D imaging. *Elife, 2017, vol. 6: e25916. doi: 10.7554/elife.25916*
69. Yi H., Strauss J.D., Ke Z., Alonas E., Dillard R.S., Hampton C.M., Lamb K.M., Hammonds J.E., Santangelo P.J., Spearman P.W., Wright E.R. Native immunogold labeling of cell surface proteins and viral glycoproteins for cryo-electron microscopy and cryo-electron tomography applications. *J. Histochem. Cytochem., 2015, vol. 63, no. 10, pp. 780–792. doi: 10.1369/0022155415593323*
70. Zhong H. Photoactivated localization microscopy (PALM): An optical technique for achieving ~10-nm resolution. *Cold Spring Harb. Protoc., 2010, vol. 2010, no. 12: pdb.top91. doi: 10.1101/pdb.top91*
71. Zhou W., Apkarian R., Wang Z.L., Joy D. Fundamentals of scanning electron microscopy (SEM). *In: Scanning Microscopy for Nanotechnology: Techniques and Applications, 2006, pp. 1–40. doi: 10.1007/978-0-387-39620-0_1*

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