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Review Article

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Study of SARS-Cov-2 using microscopy: Microscopy and its uses in challenging times

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Abstract

The humanity has witnessed a new virus called SARS-CoV-2 (also called as COVID-19) that emerged in 2019 causing the pandemic and killing millions of people throughout the world. This pandemic provided a powerful boost for the development of methods not only for diagnosis and treatment but also for basic studies on this COVID-19 virus. Microscopy is well known technique to understand the subcellular structures with great resolution. From ordinary light microscopy to advanced electron microscopy are used in the field of virology to understand its life cycle. This review provides the most recent observations made using microscopy for COVID-19 viral life cycle.

Keywords: SARS-CoV-2; Pandemic; Microscopy; Virology; RT-PCR.

Introduction

Viruses are infectious microorganisms that contain segments of nucleic acids (DNA or RNA) and are encapsulated in protein coats. Viruses cannot replicate on their own and requires a host cell/organism to multiply [1]. Viruses infect wide variety of hosts ranging from animals to humans to even other microorganisms like bacteria. There are around 219 species of viruses discovered so far that are known to infect humans. Around two third of these can also infect non-human hosts. A few species of these viruses can also possibly emerge from other mammals and birds that can cross the species barrier and infect humans [2]. A lot of pandemics in the history were witnessed by mankind due to various infections caused by microorganisms. Prominent among these pandemics include plague, cholera, flu and severe acute respiratory syndrome coronavirus (SARS CoV). Recently, the world has again witnessed another such pandemic because of new SARS CoV variant identified in 2019 that is called as COVID-19 virus [3]. To detect the infection caused by COVID-19 virus many techniques were developed and explored. Few of them include Real Time Polymerase Chain Reaction (RT-PCR), rapid antigen detection, X-ray scanning, blood testing and microscopy. Microscopy can be more informative in terms of understanding the mechanisms involved in the viral infection like entry into the host cells, replication, viral packaging, and damage to the host cells and how these viruses eventually come out of the host and spread. In this review, we will discuss various microscopic techniques with their potential merits that has been employed in understanding the COVID-19 pathogenesis.

Fluorescence microscopy technique for detection and mechanistic understanding of COVID-19

In order to design the effective therapeutic solutions, we need to have better understanding of the virus life cycle. The mechanisms that are involved in the life cycle of viruses such as its entry by attaching to host cells, its replication inside the host cell, packaging of viral particles inside the host cells and finally release of these viruses from the host cells can be understood using various imaging tools. Different types of imaging techniques were helpful to understand the pathogenesis

of COVID -19 in various cells and organs. Using the microscopy techniques, both light and electron microscopic methods, the infection of COVID -19 virus in various organs and cells have been extensively studied.

Fluorescence microscopy is a robust technology utilised in all fields of biology including virology for study of virus host interactions [4]. This microscopy depends on capturing the fluorescence signals produced by the excitation of fluorescent proteins that are coupled with the target molecules. The fluorescence proteins used for labelling the target molecules have a long history of usage in biology since the discovery of Green Fluorescence Protein (GFP) that was identified while studying the bioluminescence property of jellyfish. Since then many such fluorescence proteins have been developed using genetic engineering [5]. Fluorescence microscopy enables comprehensive examination of virus–cell interactions in fixed and live samples with great specificity. The possibility of working with live cells aids in assessing the viral structure and processes in movement in real time [4]. In addition, Fluorescence microscopy provides the advantage of sensitivity and specificity helping in assessing antibody staining and genetically encoded tags.

Some of the key findings using fluorescence microscopy include identification of possible cell entry pathways for COVID 19, the architecture and subcellular location of virus replication sites, the effects of infection on cell morphology and the timings of each of these steps in a virus-infected cell [6-8]. Other key findings include membrane fusion activity of the spike glycoprotein, subcellular localization of viral proteins and inhibitor activity [6,9-10]. Additionally, tagging COVID-19 Virus-Like Particle (VLPs) with fluorescent proteins was probable and could be useful imaging SARS-CoV-2-hACE2 internalization in host cells using for single particle fluorescence quantitative microscopy. COVID-19 VLPs could be useful tools to study the viral life cycle of COVID-19 [11].

A multi-scale fluorescence microscopy was deployed to inspect entry checkpoints of COVID-19 virus along with morphology details of the virus structure and molecular interactions with the host cell [12]. Entry of the SARS-CoV to the cells by endocytosis was investigated by fluorescence microscopy. In another study, SARS-CoV functional receptor ACE2 was labelled with GFP using stable transfection and the receptor recycling was then tracked after the cells were treated with spike proteins which mediate membrane fusion and is required for viral entry [4,13]. Fluorescence microscopy was also used to demonstrate cell–cell fusion and syncytium formation mediated by COVID-19 spike protein [4]. Another important component of the viral life cycle is the multi domain non-structural protein 3 (Nsp3) which is an essential component of the replication/transcription complex. Co-labelling immune fluorescence assays of nsp-3 or nsp-8 responsible for RNA replication showed its close association with dsRNA in infected cells. EGFP fluorescent protein was used to mark nsp3 protein involvement in pore complex formation [14,15]. To study viral assembly, plasmid constructs were engineered which permitted viral protein expression in fusion with fluorescent proteins to visualise the assembly, trafficking and release of SARS-CoV VLPs in real time [16].

Additionally, Fluorescence microscopy could also be used to study development of new in vitro cell models of COVID-19 infection. For example, combing the light-sheet microscopy and tissue optical clearing aided in creating a 3D overview of COVID-19 infection in the ferret model [17]. In addition, fluorescence microscopy enables direct visualization of single RNA molecules within single cell using fluorescence in-situ hybridization (FISH) providing unprecedented access to quantity, localization and dynamics of viral RNA [18]. CoronaFISH, the classical FISH approach adapted during 2020 to COVID-19 needs, provides a flexible, cost-efficient and versatile platform for studying COVID-19 replication at the level of single cells in culture or in tissue and can potentially be employed for drug screening and diagnosis [19].

Fluorescence microscopy has been extensively used in studying COVID-19 and related viruses. Advanced fluorescence labelling and microscopy can be utilised in the future to provide insights into COVID-19 infection. These include early labelling and tracking of viral proteins, aptamer based RNA labelling, monitoring cell physiology with fluorescent sensors and correlating fluorescence and electron microscopy techniques [4] which can potentially be used to localize COVID-19 proteins within double membrane vesicles (DMVs) and other membrane structures.

Confocal laser scanning microscopy (CLSM) technique for detection and mechanistic understanding of COVID-19

The CLSM is more advanced version of fluorescence microscopy with higher resolution, three dimensional imaging and improved signal to noise ratio features enabling capturing of fluorescence signals with high precision.

In-vitro model system, a co-culture, where the donor cells express the COVID-19 spike protein and target cells that express human angiotensin converting enzyme 2 (hACE2) receptor were studied using confocal microscopy. This revealed the hACE2 receptor and TMPRSS2 (Transmembrane serine protease 2) proteins are responsible for the cell-cell fusion and formation of syncytia among the host and donor cells. Once syncytia are formed following the recognition of the mentioned spike proteins to the host receptor endocytosis occurs and the viral membrane fuses with the endosome membrane releasing the RNA into the cytosol of the host cells [20]. The concentration of hACE2 receptor on the cells determine the frequency of the infection by COVID-19 virus [21]. Combination of confocal and super resolution imaging techniques aids in elucidating the complete reorganization of the cellular organelles like endoplasmic reticulum, peroxisomes mitochondria and secretory apparatus in the infected cells [7]. In some studies confocal were used to determine the interferons (IFNs) that are produced as antiviral response by the host cells. In case of COVID-19 infection, the virus has various proteins to inhibit the IFN production. The interaction and interference of viral proteins with IFN pathways were also studied using confocal microscopy [22]. Dual view inverted selective plane illumination microscopy (diSPIM) an improvement of confocal microscopy were used to show the pulmonary damage due to infection with submicron resolution. However, the technique was not a direct visualization of the viruses and their interactions with the host [23]. This was achieved using correlated Light Sheet Fluorescence Microscope (LSFM) with Confocal Laser Scanning Microscope (CLSM), where the LSFM was for large scale imaging and CLSM for subcellular details [24].

Electron microscopy technique for detection and mechanistic understanding of COVID-19

Electron Microscope (EM) has a history of use in the discovery of viruses. Within a few weeks of the initial outbreak, Transmission Electron Microscopy (TEM) captured the first picture of COVID-19 [25-27]. Since Electron Microscopy (EM) is an efficient method that can be used to visualize a wide range of virus-host interactions, it has long been used in the identification and understanding of viral processes. The capacity of EM to identify pathogenic agents without the need of organism-specific reagents is one of its key benefits. Even in the era of molecular diagnostics, EM is still a crucial molecular technique for quality control and for spotting new and unexpected outbreaks [28]. EM is also one of the powerful tool in microbiology, which has played significant role in diagnosis of viruses, elucidation of virus structure and function [27].

Scanning electron microscopy technique for detection and mechanistic understanding of COVID-19

Scanning Electron Microscopy (SEM) has provided immense contribution in studying three-dimensional ultrastructure of bio-specimens. SEM imaging works by capturing the different signals such as Secondary Electrons (SEs) and Backscattered Electrons (BSEs). These are the main signals used in the biological and biomedical research. SEs provide surface information of a specimen, owing to their lower energy whereas BSEs because of their high energy reaches to the deeper regions of specimen and provides depth images. SEM has shown few advantages over Transmission Electron Microscopy (TEM) in studying the virus's life cycle. One being loading multiple samples at a time and second advantage comes from the overall time taken for the samples analysis [27]. In brief, SEM is helpful in studying the three-dimensional surface topography and composition of specimens [29].

COVID-19 is associated with complex pulmonary pathology. This is mainly due to the involvement of various molecular pathways triggered by the infection [30]. There are evidences where SEM has been used in the detection of COVID-19 from the infectious patients [31]. In one of the studies the results from the SEM was compared with real-time reverse transcription-polymerase chain reaction (RT-PCR) and SEM could detect the virus in the nasopharyngeal swab samples with ct value lower than 18 [32]. SEM was used to observe size, shape, structures of viral particles and for detection of unknown microorganisms. This could be used as a potential tool in the future for any untoward outbreaks [32]. Similarly, in another study, ultra-rapid imaging SEM was used to study the COVID-19 infected Vero cells which provided detailed ultrastructural analysis of virus throughout its infectious cycle.

Evaluation of interaction between virus and host cell is necessary for development of vaccines, treatments and diagnosis. In this regard, some of the studies have used high resolution SEM and Focused Ion Beam Scanning Electron Microscopy (FIB-SEM) to determine inner cellular structure of COVID-19. Study found the evidence of cellular remodelling and the formation of specialized regions after the exposure of infection. Detection of viral particle through SEM displayed a spiky round shape with a size of 70-85 nm in diameter, which correlated with the description of COVID-19 from other studies [33,34].

Apart from COVID-19 identification, SEM has been used with other microscopy in studying the vaccines. SEM coupled with an x-ray microprobe of an Energy Dispersive Spectroscopy (EDS) helped in the evaluation of particle size, composition distribution and chemical nature of vaccines developed by companies such as Pfizer, Moderna, Astrazeneca and Janssen [35].

Transmission electron microscopy technique for detection and mechanistic understanding of COVID-19

Transmission Electron Microscopy (TEM) is most advanced microscopy in terms of magnification and resolution that is used to elucidate and characterize materials at nanoscale level. The TEM's great resolving power enabled studies of viruses at nanoscale scale level supported various research and diagnosis purposes [36]. COVID-19 analysis and elucidation of its physiopathologic mechanisms involving lungs can be imaged using TEM. Few investigations have so far characterized and linked the ultrastructural manifestations of COVID-19 infection with histopathological abnormalities. TEM has helped to discover a variety of viruses and served as a diagnostic tool for accurate identification of viruses in biological samples [37].

The novel virus, which displayed the prototypical "corona" of glycoproteins, was identified as coronavirus by micrographs which were a crucial visual validation of sequencing data. In addition, samples taken from patients during necropsy were examined using TEM to describe the endothelial and pulmonary epithelial lesions that manifested due to COVID-19. Additionally, TEM confirmed the biochemical evidences that showed virus entrance can happen via plasma membrane or endosomes [25-27].

Sequencing has been used to detect COVID-19 in samples from pneumonia patients and TEM analysis revealed that these viruses had coronavirus morphology. In order to provide morphological basis for the study of COVID-19, TEM was used to compare the morphology of HCoV-229E and COVID-19 viruses using ultrathin sections of sensitive infected cells. It was also used to further investigate the morphology of cells in Bronchoalveolar Lavage Fluid (BALF) from two ICU patients with confirmed COVID-19 infection [38]. According to reports, the COVID-19 virus enters cells via membrane fusion rather than endocytosis. Once inside the cell, the nucleocapsids assemble in the endoplasmic reticulum and mature by budding as smooth vesicles derived from the golgi apparatus. The mature virions are released when these smooth vesicles combine with cell membrane [39,40]. These events showed that COVID-19 life cycle was comparable to the SARS-CoV. These findings offer a morphological foundation for further research into the COVID-19 infection mechanism [38].

COVID-19 pathogenesis is primarily based on alveolar damage that results in epithelial cell degeneration due to necrosis, emergence of viral cytopathic effect in pneumocytes, and culminating with secondary inflammation. Other autopsy studies have demonstrated the presence of diffuse alveolar injury with the development of intra-alveolar fibrin deposits, hyaline membranes, or loosely organized connective tissue in the septal walls and alveolar gaps [41]. The other characteristics noted in this study were cytomegaly without viral inclusions, giant cell formation and desquamation of pneumocytes. These have already been described in the first SARS-associated coronavirus (SARS-CoV) outbreak [42] as well as in other viral respiratory diseases like swine-origin influenza type A [43]. Another study revealed hyperplastic type II pneumocytes with comparable cytological modifications to those found in the SARS-CoV study [44]. Furthermore, autopsies of the COVID-19 cases revealed

some similarities to those documented for H1N1 infection in humans in 2009 [45] and also with H5N1 infection. However, the COVID-19 induced alveolar damage seems to be significantly more aggressive compared to other viral infections [46].

According to Bradley et al. 2020 [41], coronavirus-like particles can be found inside tracheal epithelial cells and extracellular space or mixed with luminal mucus. The vesicles of type I and type II pneumocytes showed extensive sloughing of pneumocytes into the alveolar spaces. The Coronaviridae family, which includes enveloped viruses with surface projections frequently seen in intracytoplasmic vesicles that includes coronavirus as a member [47]. However, every virus particle's electron-microscopy structure needs to be carefully examined and compared. Other cellular structures that resemble viral structures include microvesicular bodies, clathrin-coated vesicles, segments of endosomal pathway [48] and structures with rough endoplasmic reticulum cross-sections [47]. There were instances when details were compromised when ultrastructural analysis are carried out on materials that had a number of artefacts from autolytic processes or poor fixation.

An investigation was carried out by National Institute of Virology (Indian Council of Medical Research) on the effects of temperature on COVID-19's ultrastructure. COVID-19 ultrastructures were examined using negative staining TEM to determine the type of morphological alterations observed in them with temperature fluctuation [49]. The COVID-19 strain, NIV-2020-770, which was previously isolated in this facility, was treated for 30 min. in a temperature-controlled dry bath at 2, 4, 12, 36, 45, 50, 65, and 80°C. Study showed the possible effect of temperature above 50°C on the survival of COVID-19. The noticeable outcome of the heat treatment was reduction of surface protrusion [50].

Using TEM, it was shown that COVID-19 present in enterocyte was capable of infecting human small intestinal organoids [51] and tissue samples from a rectal cancer patient who underwent surgery during the incubation period [52]. Direct COVID-19 visualisation of tissue samples form COVID-19 patient was made possible by TEM, which also offered a clear comprehension of corona virus replication in cells. In tangentially sectioned rough endoplasmic reticulum, the spikes of virus particles do resemble ribosomes, but at extremely high magnification, the two ribosomal subunits could be separated because ribosomes are much more electron-dense, rounder, and occasionally bilobated [53,47]. In this regard, when searching for COVID-19, the knowledge of electron microscopists is crucial for finding potential decoys. Secondly, evidence of COVID-19 survival in endothelial cells in subsequent samples obtained within 6 months following COVID-19 infection, while with a declining number, suggests a latent condition. The fact that COVID-19 is present in endothelial cells rather than enterocytes is also noteworthy and supports the idea that intestinal damage may result from blood dissemination rather than direct enterocyte invasion [53]. This seems to be particularly true in the case of patients with ischaemic colitis, who developed a perforation as a late complication, one month after a severe pneumonia. The presence of viral RNA and proteins does not always indicate the presence of complete and infectious particles. By using TEM, the cases described here showed for the first time that COVID-19 constructed virions were present in intestinal mucosal endothelium cells and persisted for six months following COVID-19 infection. It has been proposed that COVID-19 has a pathophysiological function in intestine injury and latent infection [54].

TEM imaging was also used to show irregular surface morphology of COVID-19 and H1N1 [55,49,56] viruses, hexagonal cross-section of HAdV [57] and smoother surface of ZIKV viruses [58,59]. In conclusion, TEM is an effective method for direct identification of virus species which records the morphology of all viral particles and counts them. It might also be applied in research for identification of antiviral medications. The process of antiviral drug suppressed viral invasion of cells could also be observed by TEM and this approach may be helpful for identifying the cellular target of viral invasion and further investigation of its pathogenesis. In addition, the analysis of the virus surface proteins using TEM can aid in the discovery and design of vaccines.

Summary

Various microscopic techniques have been in use for identification and understanding the pathophysiology of viruses (Figure 1). Each microscopic method has its own advantages and disadvantages and by careful selection, one can provide valuable insights on various stages of viral infection and replication. Light microscopy to advanced electron microscopic techniques have been employed to understand and elucidate various elements of COVID-19 virus. However, one must be careful in imaging and processing the data of microscopy in order to avoid false positive results. The techniques of sample preparation, depending on each microscopy, needs to be carefully performed and with proper negative/positive controls in order to generate repeatable and reproducible results. It is known that light microscopy among others is faster when compared with electron microscopic technique. Time taken for sample preparation for electron microscopy is higher when compared to other techniques. Microscopy in a nutshell has the advantage of providing valuable information, from single slide sample preparation, related to localization, structural analysis of viral spike proteins and its pathogenesis. Advancements in the field of microscopy can boost the understanding of viral life cycle. In this regard, correlative microscopic techniques could be a valuable addition. Combining normal light microscopy with higher resolution electron microscopy provide advantages over all the other microscopies in terms of resolution and studying the viral pathogenesis. In addition, automation is also available for imaging multiple samples along with advanced robotics. Analysis of samples through advanced programming's (use of Artificial Intelligence) would also boost the research findings in all the areas of biology and especially for quick understandings of causative microorganisms during outbreaks. We could obtain detailed information of the structure including the spikes architecture and length. In addition, obtaining multiple scans and the area covered by the automation would be of great advantage. These promising techniques needs to be tapped further for faster generation of knowledge and that will be helpful in diagnosis and treatment of infections.

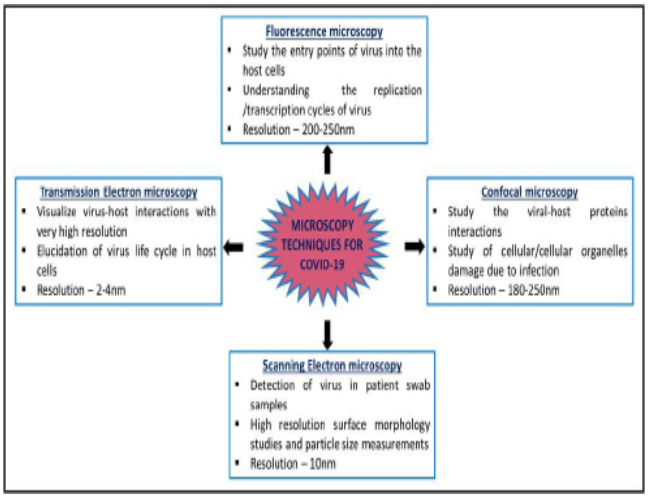

Figure 1: Schematic representation of the imaging techniques used to study the COVID-19 virus.

Declarations

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References

1. Fenner F, Bachmann PA, Gibbs EPJ, Murphy FA, Studdert MJ, et al. Structure and Composition of Viruses. Veterinary Virology. 1987; 3-19.
2. Woolhouse M, Scott F, Hudson Z, Howey R, Chase-Topping M, et al. Human viruses: Discovery and emergence. 2012; 19: 2864-2871.
3. Piret J, Boivin G. Pandemics Through out History. Frontiers in Microbiology. 2021
4. Cortese M, Laketa V. Advanced microscopy technologies enable rapid response to SARS-CoV-2 pandemic. Cell Microbiol. 2021; 23: e13319.
5. Kremers GJ, Gilbert SG, Cranfill PJ, Davidson MW, Piston DW. Fluorescent proteins at a glance. J Cell Sci. 2011; 124: 157-160.
6. Leigh KE, Modis Y. Imaging and visualizing SARS-CoV-2 in a new era for structural biology. Interface Focus. 2021; 11: 20210019 10 1098 2021 0019.
7. Cortese M, Lee JY, Cerikan B, Neufeldt CJ, Oorschot VMJ, et al. Integrative imaging reveals SARS-CoV-2-induced reshaping of subcellular morphologies. Cell Host Microbe. 2020; 28: 853-866.
8. Pahmeier F, Neufeldt CJ, Cerikan B, Prasad V, Pape C, et al. A versatile reporter system to monitor virus-infected cells and its application to dengue virus and SARS-CoV-2. J Virol. 2021; 95: 01715-01720.
9. Miorin L, Kehrer T, Sanchez-Aparicio MT, García-Sastre A. SARS-CoV-2 Orf6 hijacks Nup98 to block STAT nuclear import and antagonize interferon signaling. Proc Natl Acad Sci USA. 2020; 117: 28 344-28 354.
10. Stebbing J. JAK inhibition reduces SARS-CoV-2 liver infectivity and modulates inflammatory responses to reduce morbidity and mortality. Sci Adv. 2021; 7: 4724.
11. Gourdelier M, Swain J, Arone C. Optimized production and fluorescent labeling of SARS-CoV-2 virus-like particles. Sci Rep. 2022; 12: 14651.
12. Storti B, Quaranta P, Primio C, Clementi N, Mancini N, et al. A spatial multi-scale fluorescence microscopy toolbox discloses entry checkpoints of SARS-CoV-2 variants in Vero E6 cells. Comput Struct Biotechnol J. 2021; 19: 6140-6156.
13. Wang H, Yang P, Liu K, Guo F, Zhang Y, et al. SARS coronavirus entry into host cells through a novel clathrin- and caveolae-independent endocytic pathway. Cell Res. 2008; 18: 290-301.
14. Yuan X, Li J, Shan Y, Yang Z, Zhao Z, et al. Subcellular localization and membrane association of SARS-CoV 3a protein. Virus Res, 2005; 109: 191-202.
15. Knoops K, Kikkert M, SHEvd W, Zevenhoven-Dobbe JC, Meer Y, et al. SARS-Coronavirus Replication Is Supported by a Reticulovesicular Network of Modified Endoplasmic Reticulum. PLoS Biol. 2008; 6: 226.
16. Boson B, Legros V, Zhou B, Siret E, Mathieu C, et al. The SARS-CoV-2 envelope and membrane proteins modulate maturation and retention of the spike protein, allowing assembly of virus-like particles. J Biol Chem. 2021; 296(100111).
17. Zaeck LM, Scheibner D, Sehl J, Muller M, Hoffmann D, et al. Light Sheet Microscopy-Assisted 3d Analysis of SARS-CoV-2 Infection in the Respiratory Tract of the Ferret Model. Viruses. 2021; 13: 529.
18. Ferrer M, Henriet S, Chamontin C, Lainé S, Mougel M. From Cells to Virus Particles: Quantitative Methods to Monitor RNA Packaging. Viruses. 2016; 22: 239.
19. Rensen E, Pietropaoli S, Mueller F, Weber C, Souquere S, et al. Sensitive visualization of SARS-CoV-2 RNA with Corona FISH. Life Sci Alliance. 2022; 5: e202101124.
20. Zang R, Gomez Castro MF, Mccune BT, Zeng Q, Rothlauf PW, et al. TMPRSS2 and TMPRSS4 Promote SARS-CoV-2 Infection of Human Small Intestinal Enterocytes. Sci Immunol. 2020; 5: 3582.
21. Deroubaix Aurélie, Kramvis Anna. Imaging Techniques: Essential Tools for the Study of SARS-CoV-2 Infection. Frontiers in Cellular and Infection Microbiology. 2022; 12.
22. Yuen CK, Lam JY, Wong WM, Mak LF, Wang X, et al. SARS-CoV-2 Nsp13, Nsp14, Nsp15 and Orf6 Function as Potent Interferon Antagonists. Emerg. Microbes Infect. 2020; 9: 1418-1428.
23. Li G, Fox SE, Summa B, Hu B, Wenk C, et al. Multiscale 3-Dimensional Pathology Findings of COVID-19 Diseased Lung Using High-Resolution Cleared Tissue Microscopy. 2020.
24. Zaeck LM, Scheibner D, Sehl J, Muller M, Hoffmann D, et al. Light Sheet Microscopy-Assisted 3d Analysis of SARS-CoV-2 Infection in the Respiratory Tract of the Ferret Model. Viruses. 2021; 13: 529.
25. Bayati A, Kumar R, Francis V, McPherson PS. SARS-CoV-2 infects cells following viral entry via clathrin-mediated endocytosis. J Biol Chem. 2021; 296: 100306.
26. Ou X. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nat Commun. 2020; 11.
27. Brahim Belhaouari D, Fontanini A, Baudoin JP, Haddad G, Le Bideau M, et al. The strengths of scanning electron microscopy in deciphering SARS-CoV-2 infectious cycle. Front Microbiol. 2020; 11.
28. Goldsmith CS, Miller SE. Modern uses of electron microscopy for detection of viruses. Clin Microbiol Rev. 2009; 22: 552-563.

29. Koga D, Kusumi S, MS, Watanable T. Applications of Scanning Electron Microscopy Using Secondary and Backscattered Electron Signals in Neural Structure. Front. Neuroanat. 2021; 15.

30. Congiu T, Demontis R, Cau F, Piras M, Fanni D, et al. Scanning electron microscopy of lung disease due to COVID-19—A case report and a review of the literature. Eur Rev Med Pharmacol Sci. 2021; 25: 7997-8003.

31. Kawasaki H, Suzuki H, Furuhashi K, Yamashita K, Ishikawa J, et al. Highly Sensitive and Quantitative Diagnosis of SARS-CoV-2 Using a Gold/Platinum Particle-Based Lateral Flow Assay and a Desktop Scanning Electron Microscope. Biomedicines. 2022; 10: 447.

32. Haddad G, Bellali S, Fontanini A, Francis R, Scola B, et al. Rapid Scanning Electron Microscopy Detection and Sequencing of Severe Acute Respiratory syndrome. Coronavirus 2 and Other Respiratory Viruses. Front Microbiol. 2020; 11.

33. Caldas LA, Carneiro FA, Higa LM. Ultrastructural analysis of SARS-CoV-2 interactions with the host cell via high resolution scanning electron microscopy. Sci Rep. 2020; 10: 16099.

34. Baena V, Conrad R, Friday P, Fitzgerald E, Kim T, et al. FIB-SEM as a Volume Electron Microscopy Approach to Study Cellular Architectures in SARS-CoV-2 and Other Viral Infections: A Practical Primer for a Virologist. Viruses. 2021; 13: 611.

35. Young RO. Scanning and Transmission Electron Microscopy Reveals Graphene Oxide in CoV-19 Vaccines". Acta Scientific Medical Sciences. 2022; 6: 98-111.

36. Richert-Pggeler KR, Franzke K, Hipp K. Electron Microscopy Methods for Virus Diagnosis and High Resolution Analysis of Viruses. Front Microbiol. 2019; 9.

37. Roingeard P, Raynal PI, Eymieux S. Virus detection by transmission electron microscopy: Still useful for diagnosis and a plus for biosafety. Rev Med Virol. 2019; 29: e2019.

38. Zhao J, Zhou H, Huang W, Zhou J, Qiu M, et al. Cell morphological analysis of SARS-CoV-2 infection by transmission electron microscopy. J Thorac Dis. 2020; 12: 4368-4373.

39. Qinfen Z, Jinming C, Xiaojun H. The life cycle of SARS coronavirus in Vero. E6 cells. J Med Virol. 2004; 73: 332-337.

40. Ng ML, Tan SH, See EE. Early events of SARS coronavirus infection in vero cells. J Med Virol. 2003; 71: 323-331.

41. Bradley BT, Maioli H, Johnston R, Chaudhry I, Fink SL, et al. Histopathology and ultrastructural findings of fatal COVID-19 infections in Washington State: A case series. Lancet. 2020; 396: 320-332.

42. Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, et al. Lung pathology of fatal severe acute respiratory syndrome. Lancet. 2003; 361: 1773-1778.

43. Capelozzi VL, Parra ER, Ximenes M, Bammann RH, Barbas CSV, et al. Pathological and ultrastructural analysis of surgical lung biopsies in patients with swine-origin influenza type A/H1N1 and acute respiratory failure. Clinics. 2010; 65: 1229-1237.

44. Franks TJ, Chong PY, Chui P, Galvin L, JR RM, et al. Lung pathology of severe acute respiratory syndrome (SARS): A study of 8 autopsy cases from Singapore. Hum Pathol. 2003; 34: 743-748.

45. Nakajima N, Sato Y, Katano H, Hasegawa H, Kumasaka T, et al. Histopathological and immunohistochemical findings of 20 autopsy cases with 2009 H1N1 virus infection. Mod Pathol. 2012; 25: 1-13.

46. Ng WF, To KF, Lam WWL, Ng TK, Lee KC. The comparative pathology of severe acute respiratory syndrome and avian influenza A subtype H5N1-A review. Hum Pathol. 2006; 37: 381-390.

47. Goldsmith CS, Miller SE, Martines RB, Bullock HA, Zaki S. Electron microscopy of SARS-CoV-2: A challenging task. Lancet. 2020; 395: e99.

48. Roufosse C, Curtis E, Moran L, Hollinshead M, Cook T, et al. Electron microscopic investigations in COVID-19: Not all crowns are coronas. Kidney Int. 2020; 98: 505-506.

49. Prasad S. Transmission electron microscopy imaging of SARS-CoV-2. Indian J Med Res. 2020; 151: 241-243.

50. Walls CA, Park YJ, Tortorici MA, Wall A, McGuire AT, et al. Structure, function and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell. 2020; 180: 281-292.

51. Lamers MM, Beumer J, Van der Vaart J, Knoops K, Puschhof J, et al. SARS-CoV-2 productively infects human gut enterocytes. Science. 2020; 369: 50-54.

52. Qian Q, Fan L, Liu W, Li J, Yue J, et al. Direct evidence of active SARS-CoV-2 replication in the intestine. Clin Infect Dis. 2020; 1-6.

53. Hopfer H, Herzig MC, Gosert R, Menter T, Hench J, et al. Hunting coronavirus by transmission electron microscopy: A guide to SARS-CoV-2-associated ultrastructural pathology in COVID-19 tissues. Histopathology. 2020.

54. Martin-Cardona A, Lloreta Trull J, Albero-González R. SARS-CoV-2 identified by transmission electron microscopy in lymphoproliferative and ischaemic intestinal lesions of COVID-19 patients with acute abdominal pain: Two case reports. BMC Gastroenterol. 2021; 21: 334.

55. Goldsmith CS, Tamin A. Electron microscopic image of a negatively stained particle of SARS-CoV-2, causative agent of COVID-19. 2020.

56. Centers for Disease Control and Prevention. Images of the H1N1 Influenza Virus. CDC. 2010.

57. Ostapchuk P. The adenovirus major core protein VII is dispensable for virion assembly but is essential for lytic infection. PLoS Pathog. 2017; 13: 1006455.

58. Boigard H. Zika virus-like particle (VLP) based vaccine. PLoS Neglected Tropical Dis. 2017; 11: 0005608.

59. Sherman KE. Zika virus replication and cytopathic effects in liver cells. PLoS ONE. 2019; 14: 0214016.