Imaging SARS CoV-2 on substrates with FIB/HRSEM

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Virulent SARS-CoV-2 has been detected on stainless steel, plastic, glass and other surfaces after 3 days or longer following deposition of viral laden particles.[1] A Cu surface, on the other hand, renders the virus nonvirulent within 4-8 hrs.[2] The retention of virulent virus is particularly worrisome for public surfaces and non-disposable plastic parts of medical equipment.

We report HRSEM/FIB based techniques to examine SARS-CoV-2 on solid surfaces. Imaging of biological materials usually requires staining for contrast and substitution of solvent for water in drying. [3] Alternatively rapid freezing to avoid ice crystals, in which the water expands and breaks membranes and biological structures. None of the processes is compatible with bulk substrates. In addition, a viral structure is expected to contain very little water and room temperature in vacuum imaging is expected to be possible. We have used a Zeiss 550 SEM with a Gemini column to perform localized low kV imaging of the deactivated SARS-CoV-2 on substrates. Such is demonstrated in Figure 1. Individual virons were found to be between 100-180 nm in diameter. In addition to providing best surface sensitivity, low voltage electrons also deposit most of the electron beam energy at the surface of the material. Shown in Figure 2 is a graph of normalized energy deposited per unit volume by a 2 keV e-beam in 100 nm total thickness of PMMA, broken into five 20 nm layers, on a Si wafer vs. radial distance from an incident point exposure. The data was output from Tracer[4]. It is thought that PMMA would be a fair representative a biological material. One can see that more than an order of magnitude of energy density is deposited into the top 20 nm layer and that it is deposited within 3 nm radius of the incident beam. This is observed in Figure 3, where the total power deposited was minimized by using a low beam currents of 11 pA. The virons are comprised of a protein/lipid envelope and clusters of protein-RNA complexes within. Here it can be seen that continued scanning causes the opening of the surface viral envelope. One can begin to see the protein-RNA complexes inside the viron. We will discuss the use of beams to image deactivated SARS-CoV-2 and biological materials.

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[1] S. Riddell et al., Virol J 17: 145 (2020) https://doi.org/10.1186/s12985-020-01418-7.
[2] Z. Sun et al., J. Sus. Mat. 2214 (2020) https://doi.org/10.1016/j.susmat.2020.e00203.
[3] Lucio Ayres Caldas, Fabiana Avila Carneiro , Luiza Mendonça Higa , Fábio Luiz Monteiro ,Gustavo Peixoto da Silva , Luciana Jesus da Costa , Edison Luiz Durigon ,Amilcar Tanuri & Wanderley de Souza, Scientific Reports,10,16099 (2020).
[4] Tracer is sold by Genisys GmbH, Eschenstr. 66, D-82024 Taufkirchen (Munic) Germany.

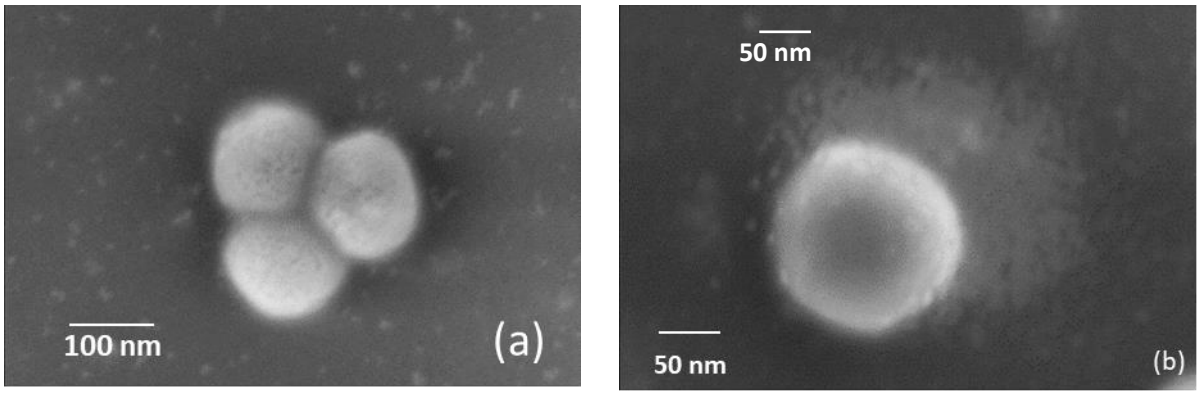

Figure 1. SEM images of SARS CoV-2 dried on Si wafer substrate. (a) Clustering and merging of virons; (b) Isolated viron.

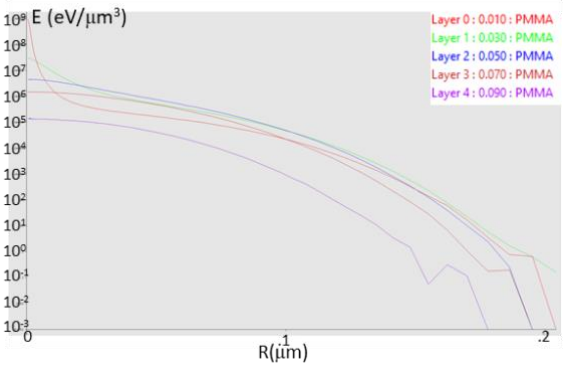

Figure 2. Energy loss per unit volume vs. radial distance from a point exposure of a 2 keV beam in 100 nm PMM on Si. Each curve represents a 20 nm layer in the 100 nm PMMA. Graph from Tracer.[7]

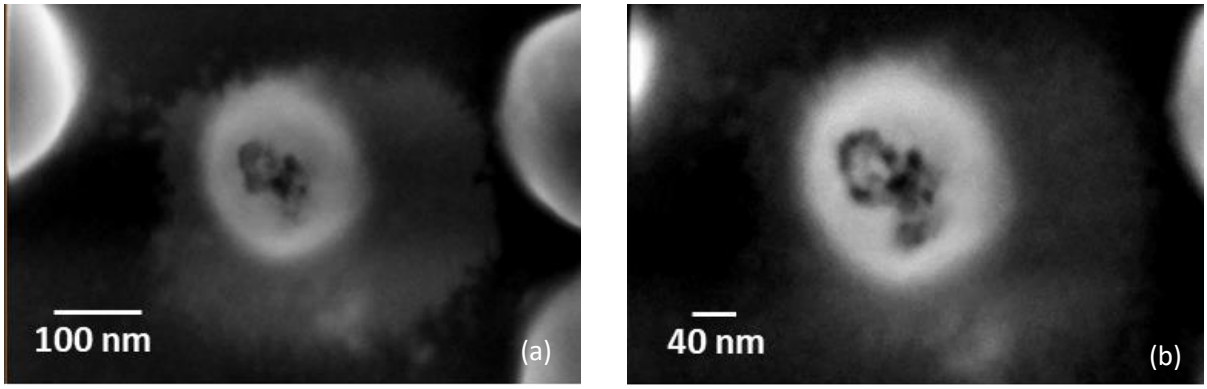

Figure 3. Sequential images of a deactivated SARS-CoV2 viron after scanning multiple times with a 2 kV, 11 pA beam.