doi:10.1017/S1431927614002748
Microsc. Microanal. 20 (Suppl 3), 2014

Zernike Phase Contrast Cryo-Electron Tomography of Bacteria and Viruses.

E. R. Wright[1, 2], R. C. Guerrero-Ferreira[1], G. Kiss[1], J. D. Strauss[1], C. M. Hampton[1]

[1.] Emory University School of Medicine, Division of Pediatric Infectious Diseases, Children's Healthcare of Atlanta, Atlanta, Georgia
[2.] Robert P. Apkarian Integrated Electron Microscopy Core, Emory University, Atlanta, Georgia

Cryo-electron tomography (cryo-ET) is currently the only method available through which pleomorphic biological specimens are imaged to produce three-dimensional (3D) structures at ~2 to 10 nm resolution [1]. There remain challenges associated with imaging intact bacteria and pleomorphic viruses by cryo-ET. In particular, investigators cope with the low contrast of cryo-preserved, unstained specimens and the requirement for low dose imaging in order to limit radiation damage to the specimen. Zernike phase contrast (ZPC) transmission electron microscopy (TEM) was developed to overcome these and other complications [2]. Here we present results showing the success of using ZPC technologies for cryo-ET investigations of bacterial cells and pleomorphic viruses.

Aliquots of bacterial cells and purified viruses were flash frozen onto glow-discharged Quantifoil carbon grids in liquid ethane with a FEI Mark III Vitrobot. Cryo-EM and cryo-ET data collection was performed with a JEOL JEM-2200FS 200 kV FEG-TEM equipped with the Zernike phase plate airlock system located in the back-focal plane of the objective lens, an in-column energy filter (slit width 20 eV), a cryo-transfer specimen holder (Model 914, Gatan), and a 4k x 4k Gatan Ultrascan CCD camera. Images were acquired with a pixel size ranging from 0.55 to 1.1 nm on the specimen. For tilt series, a total electron dose between 30 $e^-/Å^2$ and 120 $e^-/Å^2$ was fractionated over tilt series ranging from -62° to +62°. Tilt series images were taken automatically with 2° tilt increments by using Serial EM [3].

We explored the potential of ZPC cryo-ET for the structural characterization of bacteria and viruses by analyzing data of *Caulobacter crescentus* and *Vibrio vulnificus* cells as well as several pleomorphic viruses, such as respiratory syncytial virus (RSV), HIV-1, and influenza, collected under both DPC and ZPC conditions at total electron doses of between ~30 $e^-/Å^2$ and ~120 $e^-/Å^2$. In our analysis of the ZPC data of RSV, we resolved the ordered arrangement of the M2-1 protein linkage between the matrix protein and the ribonucleoprotein (RNP) complex (Fig 1). We also noted better definition to the structure of the RNP when compared to conventional DPC data of RSV (Fig 1). In addition, we demonstrated that the thin carbon film ZPC technology allows one to reduce the electron dose applied to the specimen and maintain high contrast; achieve higher contrast in each tilt series image and in the final 3D reconstruction; improve image alignment due to the greater SNR of each image and the reduction of fiducial displacement; and reduce noise in the 3D volume [4]. Future studies will be directed at improving methods for ZPC cryo-ET data collection and analysis and probing the now accessible structures in bacteria and viruses.

References:
[1] JL Milne, S Subramaniam, Nature Reviews. Microbiology **7(9)** (2009), p. 666-675.
[2] R Danev, K Nagayama, Ultramicroscopy **88** (2001), p. 243-252.
[3] JR Kremer, DN Mastronarde, and JR McIntosh, Journal of Structural Biology **116(1)** (1996), p. 71-76.
[4] RC Guerrero-Ferreira, ER Wright, Journal of Structural Biology **185(1)** (2014), p. 129-133.

[5] This research was supported by funds from Emory University, Children's Healthcare of Atlanta, the Emory Center for AIDS Research, the Georgia Research Alliance, Human Frontiers Science Program, National Institutes of Health (1R01GM104540 and 1R21AI101775), and the National Science Foundation (0923395) to E.R.W. Cryo-EM/ET data was collected at the Emory University Robert P. Apkarian Integrated Electron Microscopy Core.

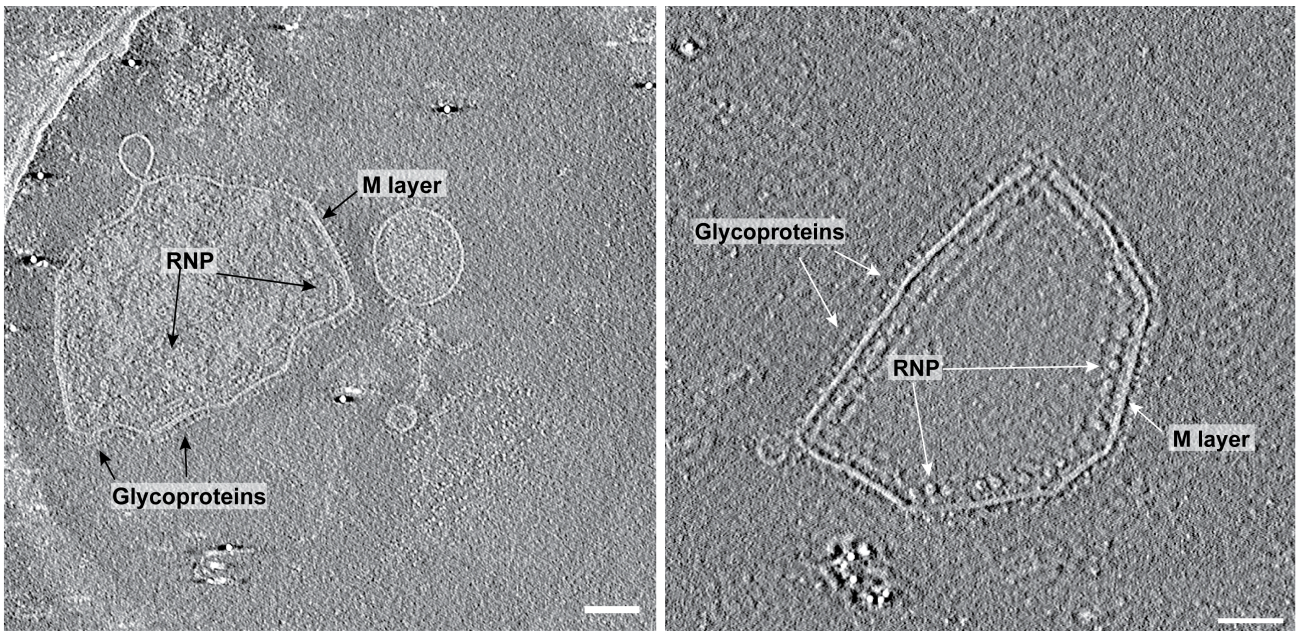

Figure 1. DPC and ZPC cryo-ET of frozen-hydrated respiratory syncytial virus (RSV). Slices (7.6 nm) from DPC (left) and ZPC (right) three-dimensional reconstructions (tomograms) of RSV (glycoproteins, matrix (M) protein layer, and ribonucleoprotein (RNP) complex are identified). Scale bars, 100 nm.

https://doi.org/10.1017/S1431927614002748 Published online by Cambridge University Press