Application Note

Poseidon™

Visualizing Viral Assemblies Using *In Situ* Liquid TEM

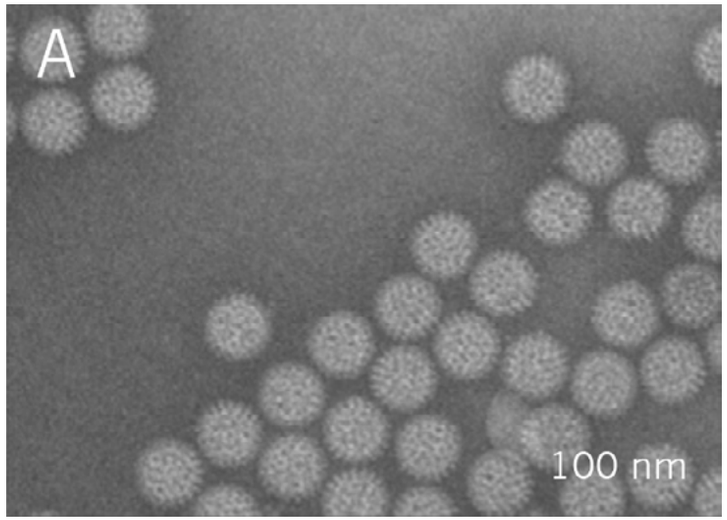
Introduction

In this experiment, rotavirus particles were imaged in solution using a Poseidon *in situ* liquid TEM holder. The obtained images were used to calculate the first high resolution, 3D reconstructions of biological assemblies from single particles contained entirely within liquid. Distinct structural subpopulations of particles contained in liquid were observed, in contrast to the homogeneous structural states of chemically fixed and ice-embedded specimens.

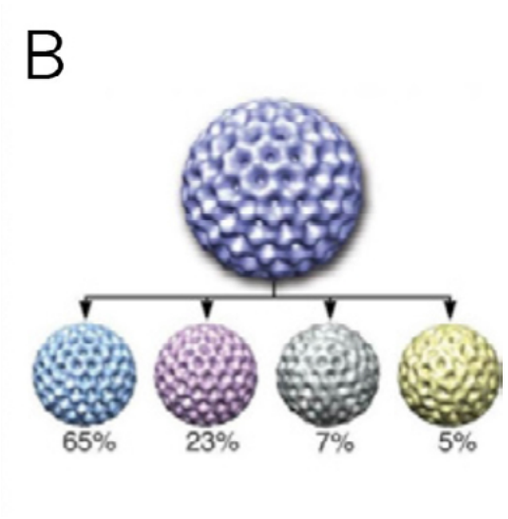
Experiment

All experiments were conducted in Deborah Kelly's lab at the Virginia Tech Carilion Research Institute. The surface of one Poseidon E-chip™ was functionalized with a lipid monolayer (Affinity Capture) containing polyclonal antibodies against VP6 (a protein present in rotavirus). A solution containing rotavirus (0.1 mg ml^{-1}) and a low concentration of contrast reagent (0.2% w/v uranyl formate) was applied to the functionalized E-chip. The quantity of staining reagent was not sufficient to cause fixation the rotavirus particles and was included to enhance contrast for downstream image processing. Images of rotavirus particles contained in a 150 nm liquid layer (Image A) were recoded using an FEI Spirit BioTwin TEM equipped with a tungsten filament operating at 120 kV under low-dose conditions (~5 electrons/ $Å^2$ for each exposure). 3D reconstructions of both the *in situ* and vitreous ice embedded specimens were generated using the software, RELION, from 600 and 572 particles, respectively. The projection averages of the *in situ* and frozen specimens are shown in Figure 1.

Discussion



A single 3D volume average with a resolution of 25 Å was calculated from a population of 600 particles, and is shown in Image B (purple). The majority of the rotavirus particles were classified into two subpopulations: indicated in blue (65%) and pink (23%). Two additional reconstructions contained only 7% (gray) and 5% (yellow) of the total particles present in the image stack. The presence of four distinct subpopulations of the particles indicate that they exhibited some degree of structural heterogeneity, as they were not fixed, only tethered to the surface



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Reference: For further information about the studies described here please refer to: Lab Chip, 2013, 13, 216-219

of the liquid chamber. In contrast to what was found for the *in situ* specimens, a single, statistically significant population was present in the image stack of cryo-TEM prepared samples.

Conclusion

These results demonstrate the first example of a 3D reconstruction of biological species in their native liquid environment. The authors report a ~25 Å resolution obtained after averaging 600 rotavirus particles, which was comparable to the resolution (~24 Å) of the same sample using traditional cryo-TEM imaging. A greater degree of structural heterogeneity was observed among virus particles when imaged in solution, rather than in vitrified ice. This heterogeneity, or dynamic subpopulations, may be a result of Brownian or beam-induced motion. Although motion is likely suppressed to a higher degree when immobilized in vitreous ice, tethering particles to the E-chip surface via Affinity Capture enables visualization of the dynamic sub-states, which are absent when the particles are immobilized via chemical fixation or freezing.

Applications

Poseidon is compatible with a broad range of materials and biological samples and enables the user to achieve nanometer to atomic resolution imaging of specimens in dynamic liquid environments. The Poseidon platform is compatible with both TEM and STEM and is ideal for correlative light and electron microscopy (CLEM) studies. The consumable, Poseidon E-chip devices, which are used to form the sample chamber, are fully compatible with standard sterilization and tissue culture techniques, thus cells may be grown directly on the E-chip surface for cellular imaging, labeling, or nanoparticle uptake studies. The Poseidon TEM holder is available with either 2 or 3 liquid ports and can be easily configured on an experiment-by-experiment basis for flow, mixing or static operation. Thus in addition to maintaining a hydrated environment, dynamic processes such as nucleation, nanoparticle growth, self-assembly, and particle-particle interactions can be observed. Contact us to discuss Poseidon's full range of capabilities. We can be reached at (919) 377-0800 or contact@protochips.com.

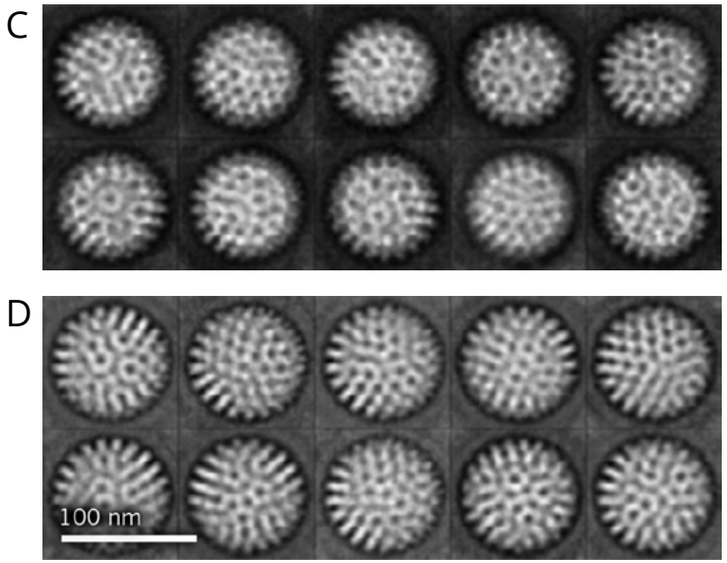

Figure 1: Projection averages of rotavirus particles calculated from (C) *in situ* and (D) frozen specimens. Contrast of the ice averages (D) is inverted for ease of comparison.

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