

# Frederick National Laboratory for Cancer Research

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*sponsored by the National Cancer Institute*

## National Cryo-Electron Microscopy Facility User Report

**February 29, 2024**



Project Number:  
NCEF-086-010-10564

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## Project Description

Epstein-Barr virus (EBV) is an oncogenic virus responsible for ~2% of human cancers. The dyad symmetry element (DS) of the EBV origin of replication contains two pairs of binding sites for the EBV protein EBNA1 and three nonamer TTAGGGTTA sites, all of which are necessary for proper replication of the EBV genome. Our lab previously published the structure of the ½ DS bound to two homodimers of the EBNA1 DNA binding domain, but the shelterin components TRF2 and Rap1 have also been shown to interact with the DS and to play roles in EBV replication, and TRF2 canonically binds to telomeric TTAGGG repeats via its own DNA binding domain. Here we propose to elucidate the structure of the full DS bound to recombinant near-full-length EBNA1, full length TRF2, and full length Rap1, to better understand the structural basis for EBV genome replication.

## Grid Inventory and Handling

**Grids clipped and loaded into microscope:** Loaded three grids from one box: three pre-clipped grids from Box 1 (1-1, 1-2, 1-3).

Grid Name	Clipping condition	Screening report
1-1	Previously clipped	~99% opaque. Not imageable.
1-2	Previously clipped	Significant grid square cracking and tearing. Many imageable areas. Good particle distribution and concentration, but significant ice contamination at high magnification, which may interfere with particle picking. Imageable, but not recommended.
1-3	Previously clipped	More intact than 1-2. Many imageable areas. Some areas with significant crystalline ice; other areas with no crystalline ice. Little to no ice contamination at high magnification. Good distribution and concentration of particles. Imageable. <b>SOLE GRID IMAGED.</b>

## Sample quality and particle concentration

The first grid was mostly opaque and not imageable. However, the other two grids had little to no opaque areas, leaving many potential imageable areas on each grid. While these imageable areas were reduced on each grid—by cracking and tearing on grid 1-2, and crystalline ice on parts of grid 1-3—each grid still had plenty of imageable areas available.

However, grid 1-2 also had significant ice contamination at high magnification, which can interfere with particle picking and impact the quality of the dataset. For this reason, grid 1-2 is not recommended for imaging.

Grid 1-3, on the other hand, had little to no ice contamination in high-magnification screening images, and so grid 1-3 was selected for data acquisition for this imaging session. If more data is needed for this sample, we recommend sending additional grids.

The 2D classification (NCEF500) shows the number of particles per image ranges from ~0 to ~250, with a peak at ~0 particles per image, with a slight secondary peak at ~75 and a gradual taper down from around ~100 to around ~200 particles per image. The particles are averaging to multiple classes, which may represent multiple views.

## Notes on data collection

Post-GIF, on-camera dose ranged from 27-47  $\text{e}^-/\text{\AA}^2$ , and the total sample dose remained 50  $\text{e}^-/\text{\AA}^2$ .

## Recommendation on improving sample quality

The significant high-magnification ice contamination on grid 1-2 could impact particle picking and the data quality of the dataset, especially when compared to grid 1-3, which showed little to no ice contamination at high magnification during screening.

Grid 1-2 does have many imageable areas available, and particles are present in screening images, and so grid 1-2 is technically imageable, and would remain available for a future imaging session should it be needed. However, due to the higher levels of contamination at high magnification, grid 1-2 is not recommended for imaging. If more data is needed for this sample, we do recommend sending additional grids—especially if the ice contamination can be reduced, producing grids more similar to grid 1-3.

As always, continuing to use proper grid handling techniques to mitigate both crystalline ice and grid square cracking or tearing should continue to ensure many imageable areas are available in future batches of grids, as well.

## Grid storage and recommendation for further use

Grids were stored in box 34-12 (1-1, 1-2, 1-3) and are scheduled for destruction on May 29<sup>th</sup>, 2024, unless flagged for another imaging session. Should more data be needed for this sample, we recommend sending additional grids, while grid 1-2 will also remain available for a future imaging session if needed. For more details, see previous section.

## Quality Control Notes

Resolution limits and motion are acceptable for the sample ice thickness that was observed, with resolution ranging from 3-10  $\text{\AA}$  and a peak at 6.0  $\text{\AA}$ . The ice thickness measurements ranged from 15 nm to 150 nm, with a small initial peak at 15 nm and a main, primary peak around ~115 nm. Astigmatism values ranged from 0 nm to 100 nm with a peak at 30 nm. Defocus measurements ranged from -0.9  $\mu\text{m}$  to -3.6  $\mu\text{m}$ , with a peak at -2.4  $\mu\text{m}$ .

## Microscope State

<b>Imaging Mode:</b> Nanoprobe EFTEM	<b>Camera:</b> K3
<b>Camera Mode:</b> Counting	<b>Physical Pixel Size:</b> 5 $\mu\text{m}$

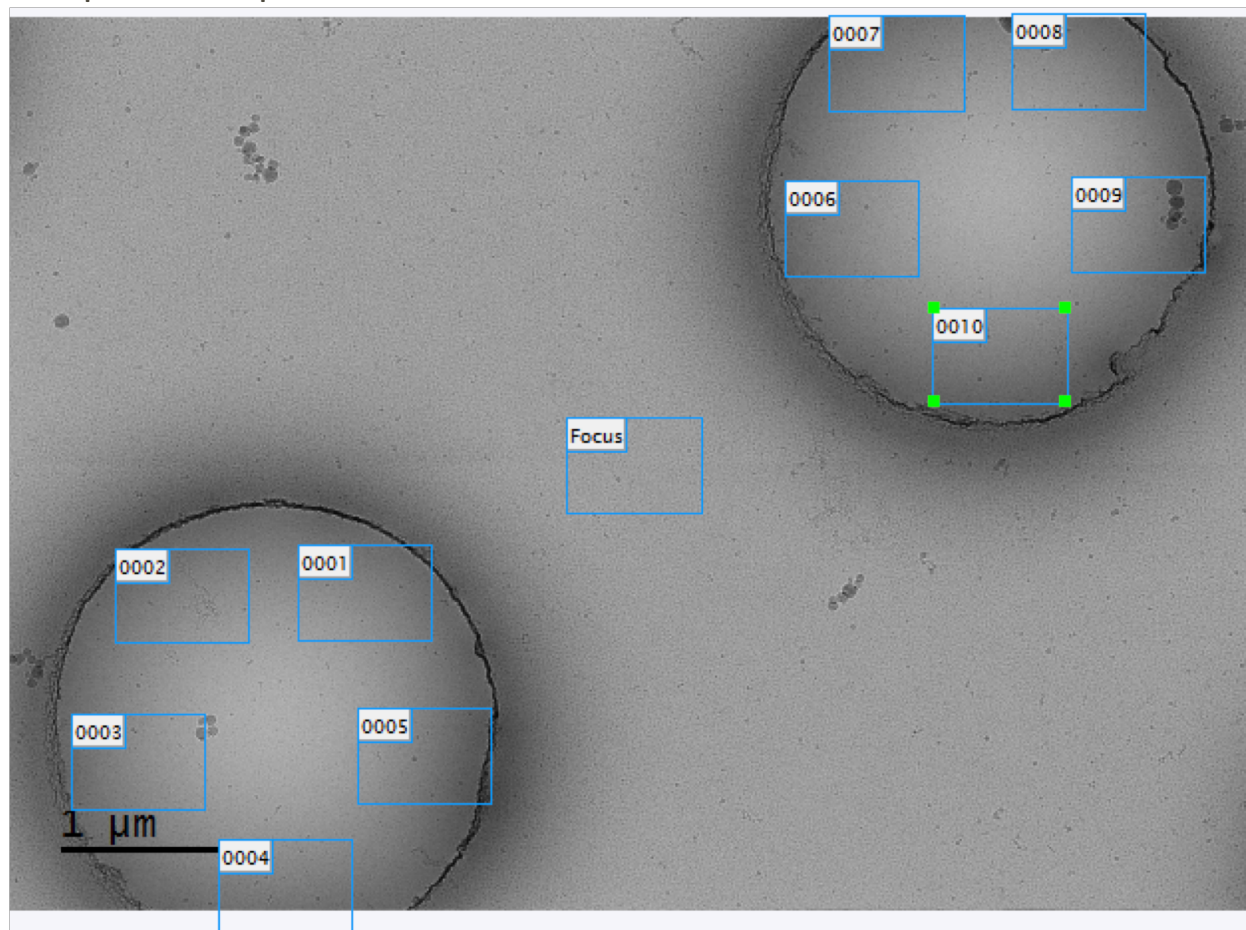
<b>Camera Configuration:</b> 180°, flip about vertical axis		
<b>C1 Aperture:</b> 2000 $\mu\text{m}$	<b>C2 Aperture:</b> 100 $\mu\text{m}$	<b>Objective Aperture:</b> None
<b>Select Area Aperture:</b> None	<b>Cs:</b> 2.7 mm	<b>Microscope:</b> Blue

## Imaging States

### Data Acquisition State

<b>Magnification:</b> 81,000	<b>Image Pixel Size:</b> 1.07 $\text{\AA}$	<b>Nominal Dose:</b> 50.0 $\text{e}^-/\text{\AA}^2$
<b>Spot Size:</b> 7	<b>Illuminated Area:</b> 925.3 nm	<b>Dose rate:</b> 26.11 $\text{e}^-/\text{s}/\text{phys. pixel}$
<b>Binning:</b> 1	<b>Camera Mode:</b> Counting	
<b>Exposure Time:</b> 2.2 sec	<b>Number of Frames:</b> 40	<b>Settling Time:</b> 1 sec
<b>Defocus Range:</b> -1.0 to -2.5 $\mu\text{m}$	<b>Defocus Step:</b> 0.25 $\mu\text{m}$	<b>Focus Interval:</b> 12 $\mu\text{m}$
<b>Program:</b> Latitude	<b>Acquisition Method:</b> Single Particle	
<b>Number of Exposures:</b> 10,709	<b>Dark Reference Interval:</b> Every 2 hours, automatic	
<b>Energy Filter:</b> Yes	<b>Filter Slit:</b> 20 eV	<b>ZLP Alignment Interval:</b> 4 hr, automatic
<b>Imaging Strategy:</b> 5 images per hole, 10 images per template, using image shift per target.		

### Template Setup:



## Focus State

<b>Magnification:</b> 81,000	<b>Image Pixel Size:</b> 1.07 Å	<b>Nominal Dose:</b> N/A
<b>Spot Size:</b> 7	<b>Illuminated Area:</b> 925.3 nm	<b>Exposure Time:</b> 1.0 sec
<b>Binning:</b> 1	<b>Camera Mode:</b> Counting	

## Hole State

<b>Magnification:</b> 8,700	<b>Image Pixel Size:</b> 1.03 nm	<b>Nominal Dose:</b> < 0.5 e <sup>-</sup> /Å <sup>2</sup>
<b>Spot Size:</b> 7	<b>Illuminated Area:</b> 9.013 μm	<b>Exposure Time:</b> 1.0 sec
<b>Binning:</b> 1	<b>Camera Mode:</b> Counting	

## Grid State

<b>Magnification:</b> 580	<b>Image Pixel Size:</b> 15.8 nm	<b>Nominal Dose:</b> N/A
<b>Spot Size:</b> 7	<b>Illuminated Area:</b> 201.4 μm	<b>Exposure Time:</b> 1.5 sec
<b>Binning:</b> 1	<b>Camera Mode:</b> Counting	

## Atlas State

<b>Magnification:</b> 135	<b>Image Pixel Size:</b> 66.6 nm	<b>Nominal Dose:</b> N/A
<b>Spot Size:</b> 7	<b>Illuminated Area:</b> 1015 μm	<b>Exposure Time:</b> 0.5 sec
<b>Binning:</b> 1	<b>Camera Mode:</b> Counting	

## Microscopist:

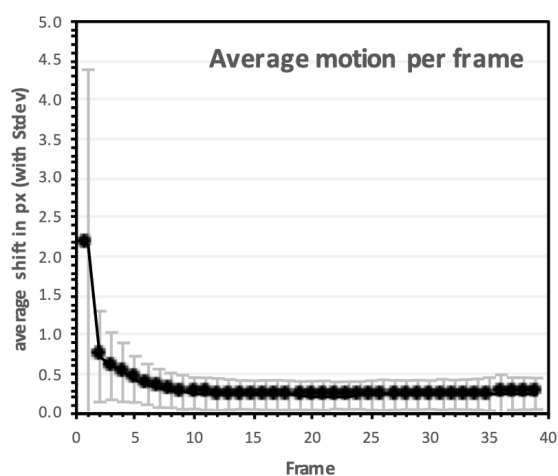
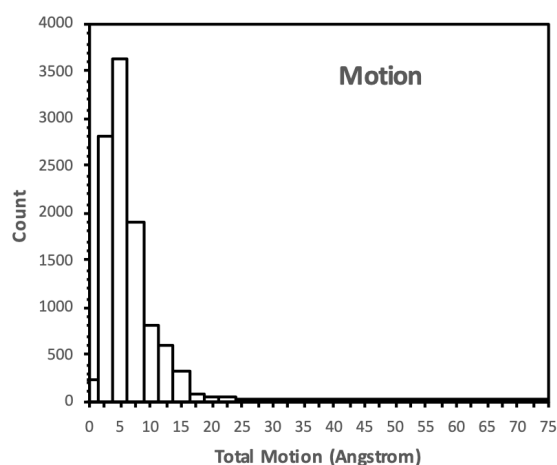
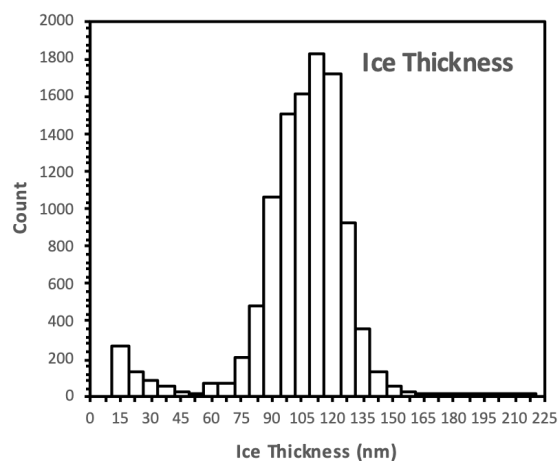
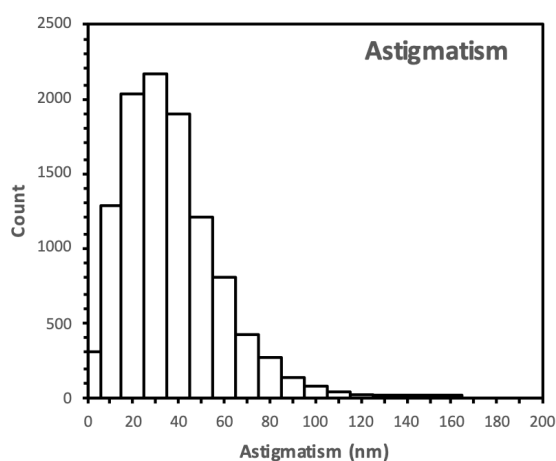
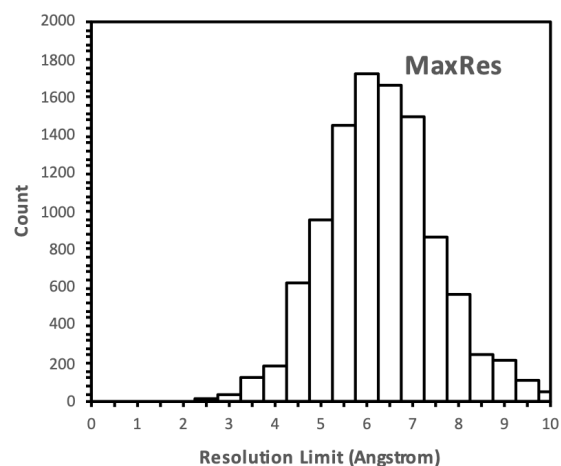
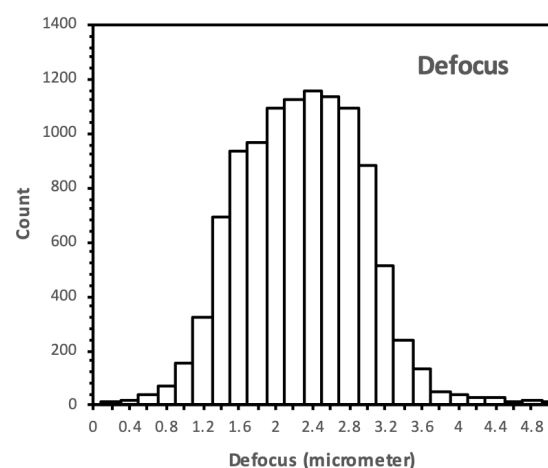
Tara L. Fox

## Acknowledgment

\*Note: In any publications and posters that use data provided by NCEF, we require that you include acknowledgment of, “This research was, in part, supported by the National Cancer Institute’s National Cryo-EM Facility at the Frederick National Laboratory for Cancer Research under contract 75N91019D00024”.

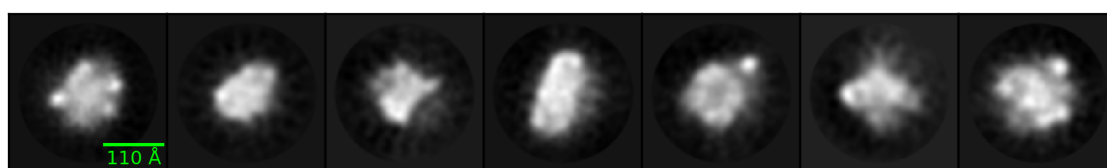
Thank you for choosing NCEF for your imaging project.

# Quality Control

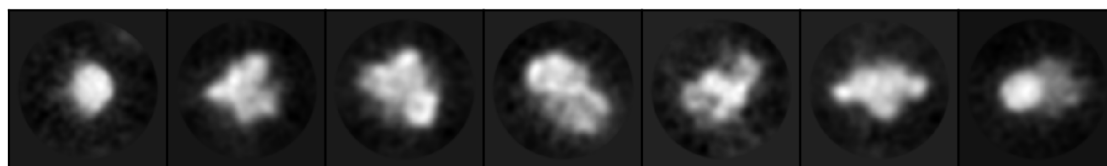


Movies are motion corrected globally with MotionCor2 and ctf fitting is done with CTFFIND4. Average ice thickness can be determined on images only when the GIF is being used. In that case it is determined with using the inelastic mean free path of  $\Lambda = 250$  nm determined on our system. An application note on ice thickness determination is available upon request.

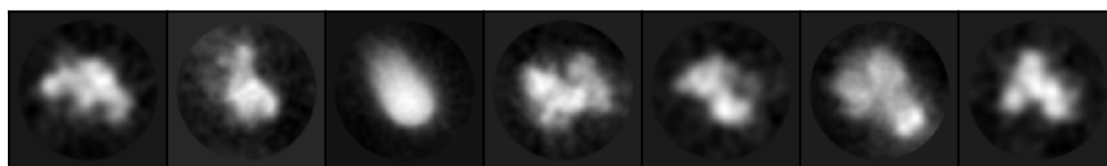




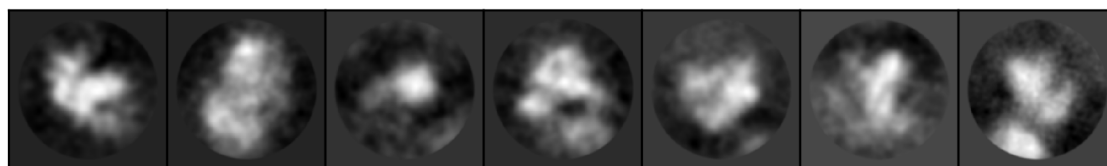
6713 5691 3734 3102 2677 2360 2308



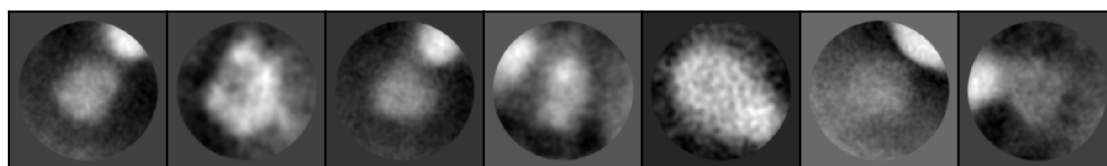
2246 1752 1645 1596 1329 1297 1248



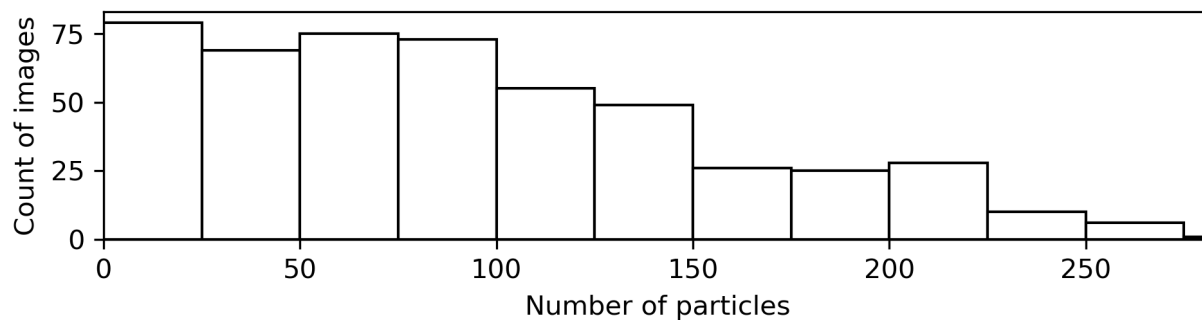
1044 1040 997 819 725 677 653



646 359 318 303 201 183 175



119 117 76 40 25 15 13



Total particles picked: 50757  
 Particles included in 2D classes: 46260

NCEF 500



The first 500 images from the dataset were put through 2D classification solely with the purpose of quality control of the sample. No further processing of the data was done. Motion correction and CTF estimation were performed using MotionCor2 and ctffind4, respectively. Particle picking was done with crYOLO using the pre-trained general model available from <https://sphire.mpg.de/2D> classification was performed in two rounds with RELION, using cinderella with a custom-trained model to select classes between rounds.