



	Experiment title: Structural studies of the helicase/primase complex from Herpes simplex virus	Experiment number: MX-2230
	Beamline: CM01	Date of experiment: from: 15/11/2019 to: 18/11/2019
Shifts: 6	Local contact(s): Michael Hons	<i>Received at ESRF:</i>
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Report:

Herpes simplex virus (HSV) encodes seven proteins necessary for viral DNA synthesis, including a helicase/primase complex - UL5/UL8/UL52. Despite the high interest, all the attempts to obtain the detailed structure of this complex have been unsuccessful to date. The aim of this project is to use cryo-EM technique to reveal the structure of the helicase/primase complex and to clarify the details of interaction of proteins within. Unfortunately, we have lost our sample grids during the transportation to ESRF. We were able to prepare new grids (Lacey) on-spot courtesy of ESRF, based on our preliminary estimated sample preparation protocol. Using these grids Cryo-EM data were finally collected at the ESRF instrument equipped with K2 detector and processed as described further.

Table 1. Data acquisition and processing statistics

Data Acquisition			
Microscope parameters		Initial data parameters	
Magnification	130000x	Pixel size, Å	1.053
Nominal defocus range, µm	[-0.5, -2.6]	Number of frames	4148
Defocus step, µm	0.2	Frame rate	40
Exposure Time, s	7		
Total dose, e ⁻ /Å ²	41.4		
Dose per frame, e ⁻ /Å ²	1.03		
Voltage, kV	300		
Data processing			
Used software		Resolution	
Motion correction	WARP 1.0.7	Total number of particles for classification	238000
CTF estimation	WARP 1.0.7	Total number of particles for final refinement	55600
Particle picking	WARP 1.0.7	Resolution (FSC _{0.143}), Å	6.5*

Classification and refinement	cryoSPARC 2.12.4
Local resolution estimation	cryoSPARC 2.12.4

* According to cryoSPARC

Example of a good image obtained from GridSquare_15446072 is shown on Fig. 1a. Obtained images were processed as described in Table 1.

Fig. 1a. Helicase-primase complex' particles are highlighted by blue circles.

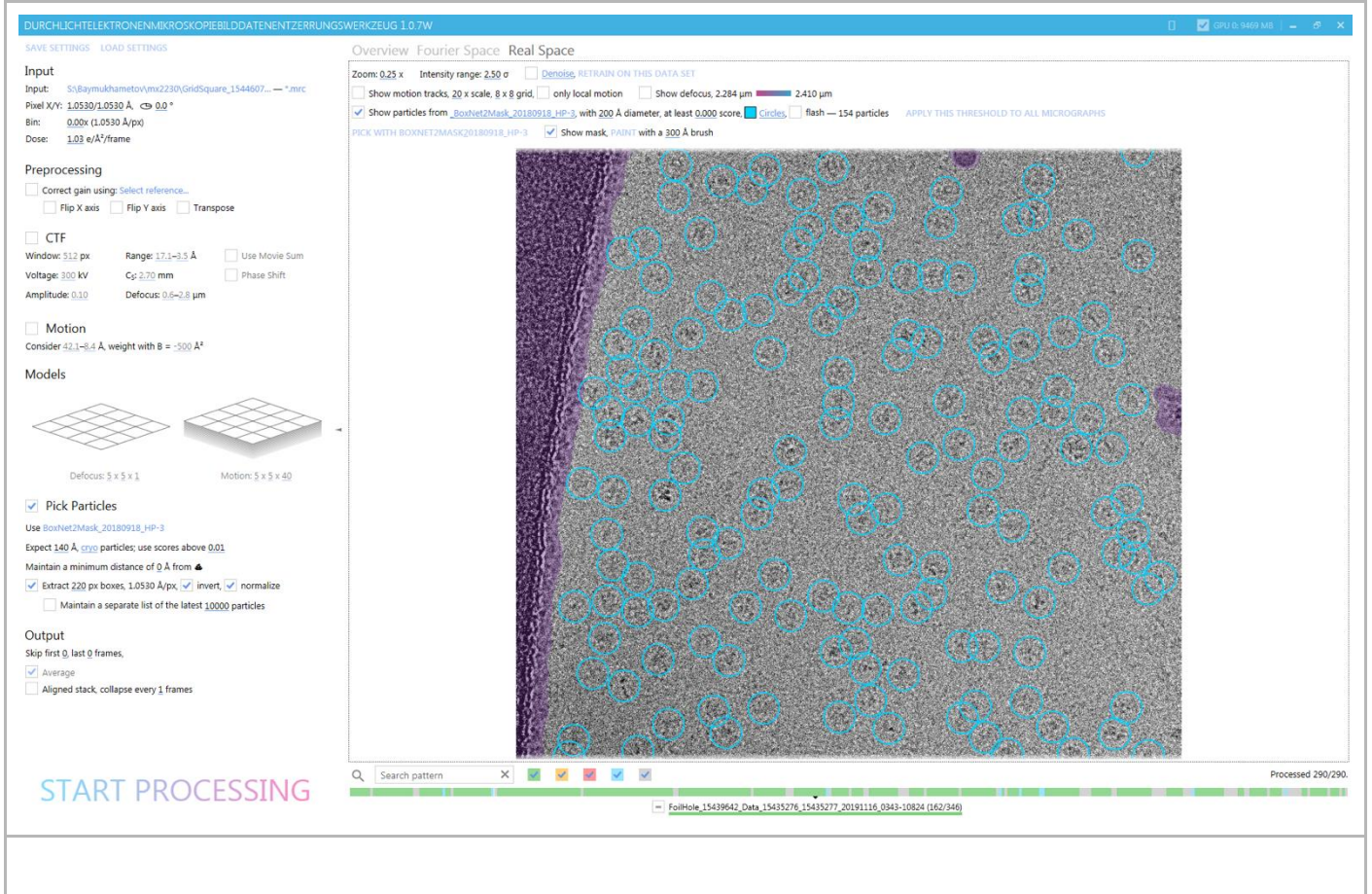
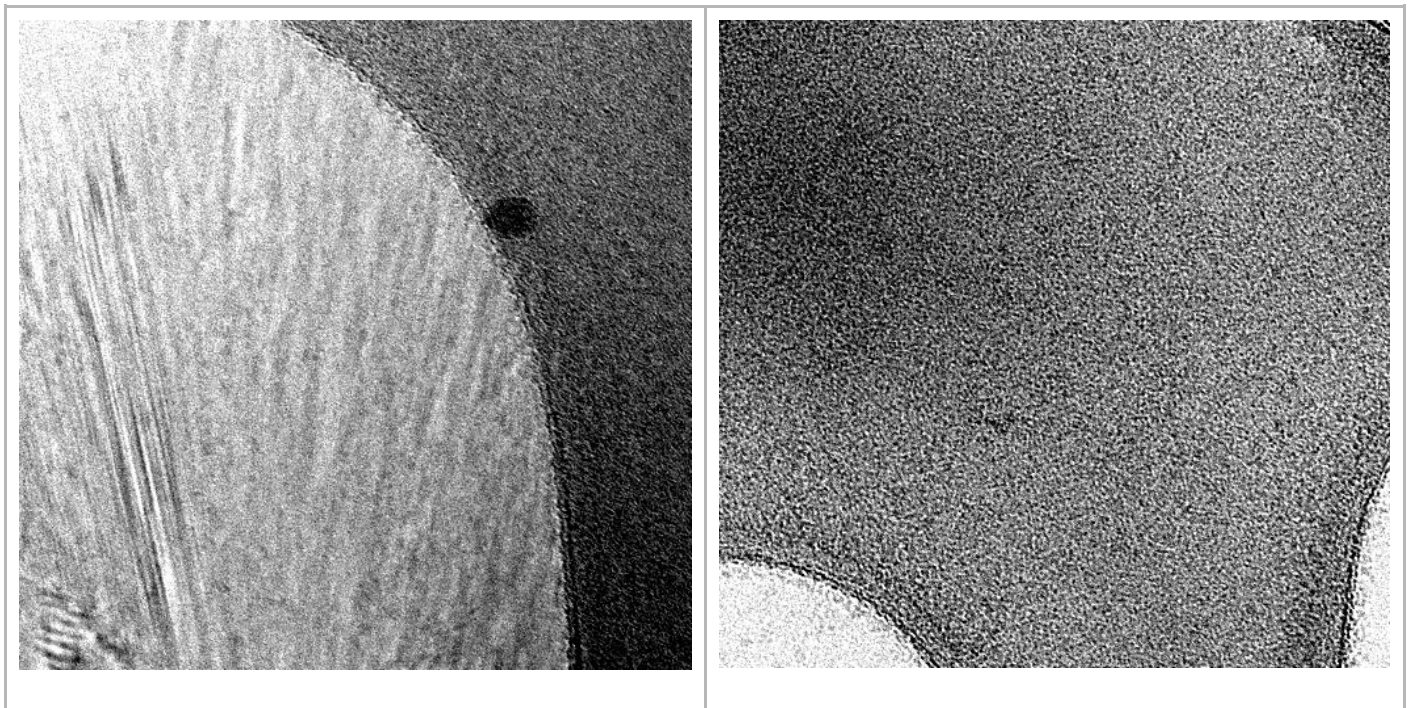


Fig. 1b. Example of images excluded from data processing pipeline (GridSquare_15446072) containing crystal ice or carbon.



Data processing

The raw data was pre-processed using WARP (Tegunov D. et al., 2019). The standard neural network BoxNet2Mask_20180918 was retrained using three consecutive rounds based on a training sample of 50 images. The dataset had a large proportion of unsuitable for further processing images, which contain areas of crystalline ice and carbon (Fig. 1b) and were manually excluded at the pre-processing stage. Images for which it was not possible to approximate the CTF were also excluded from processing. Statistics are presented in Table 2. Distribution of defocus values, astigmatism as well as resolution estimation for 2773 selected images are shown on Fig. 2.

Table 2. Data pre-processing statistics

Grid Square	Quality, %		All images	Good images (after retraining)	Particles (after retraining)	Avg. particles per image (after retraining)
GridSquare_15445564	Carbon / crystalline ice	70	274	87	6566	80
GridSquare_15445584	Carbon / crystalline ice	40	290	181	4598	30
GridSquare_15445996	Carbon / crystalline ice	20	238	181	17825	100
GridSquare_15446000	Carbon	20	301	231	20018	90
GridSquare_15446006	Carbon	10	42	38	1885	50
GridSquare_15446072	Carbon / crystalline ice	20	346	287	25567	90
GridSquare_15446076	Carbon / crystalline ice	40	324	182	18051	100
GridSquare_15446078	Carbon / crystalline ice	50	231	121	11689	100
GridSquare_15446082	Carbon	40	200	114	5860	50
GridSquare_15446084	Carbon / crystalline ice	30	282	192	21976	110
GridSquare_15446088	Carbon / crystalline ice	50	303	152	13535	90
GridSquare_15446092	Carbon / crystalline ice	20	331	256	23664	90
GridSquare_15446094	Carbon / crystalline ice	20	279	218	21108	100
GridSquare_15446096	Carbon	30	219	164	12439	80
GridSquare_15446103	Carbon	10	275	243	24533	100
GridSquare_15446105	Carbon	40	213	126	8264	70
Total or Average		30	4148	2773	~238000	80

2D classification of about 238 000 picked by WARP particles from 2773 selected images was made with cryoSPARC v2 in two stages (Fig. 3) with the following *ab-initio* reconstruction and 3D classification (Heterogeneous refinement) to assign selected 2D projections to appropriate 3D structures and finally get rid of false positive particles. Subsequently, refinement procedure was made for each of subsets of particles. This led to electron densities for the protein complex with an average resolution about 6.5Å (Fig. 4,5).

To summarise, data analysis revealed two problems:

1. The sample has preferential orientations (Fig.4, bottom view), which limiting us to get all the needed views and thus impeding from structure reconstruction (major problem).
2. It seems that there is a problem of abundance of part of the structure (Fig.3,5) – a flexible part (minor problem).

Despite these facts, we are planning to improve the sample preparation protocol to eliminate preferential orientations of the particles (detergents, other types of grids, etc). We are also going to try a helicase-primase

complex with inhibitor, which might fix the complex in one state. We are positive that those actions could lead to improved grids, which can be used in a new Cryo-EM experiment at ESRF.

Figure 2. Major dataset parameters estimated per partical by WARP.

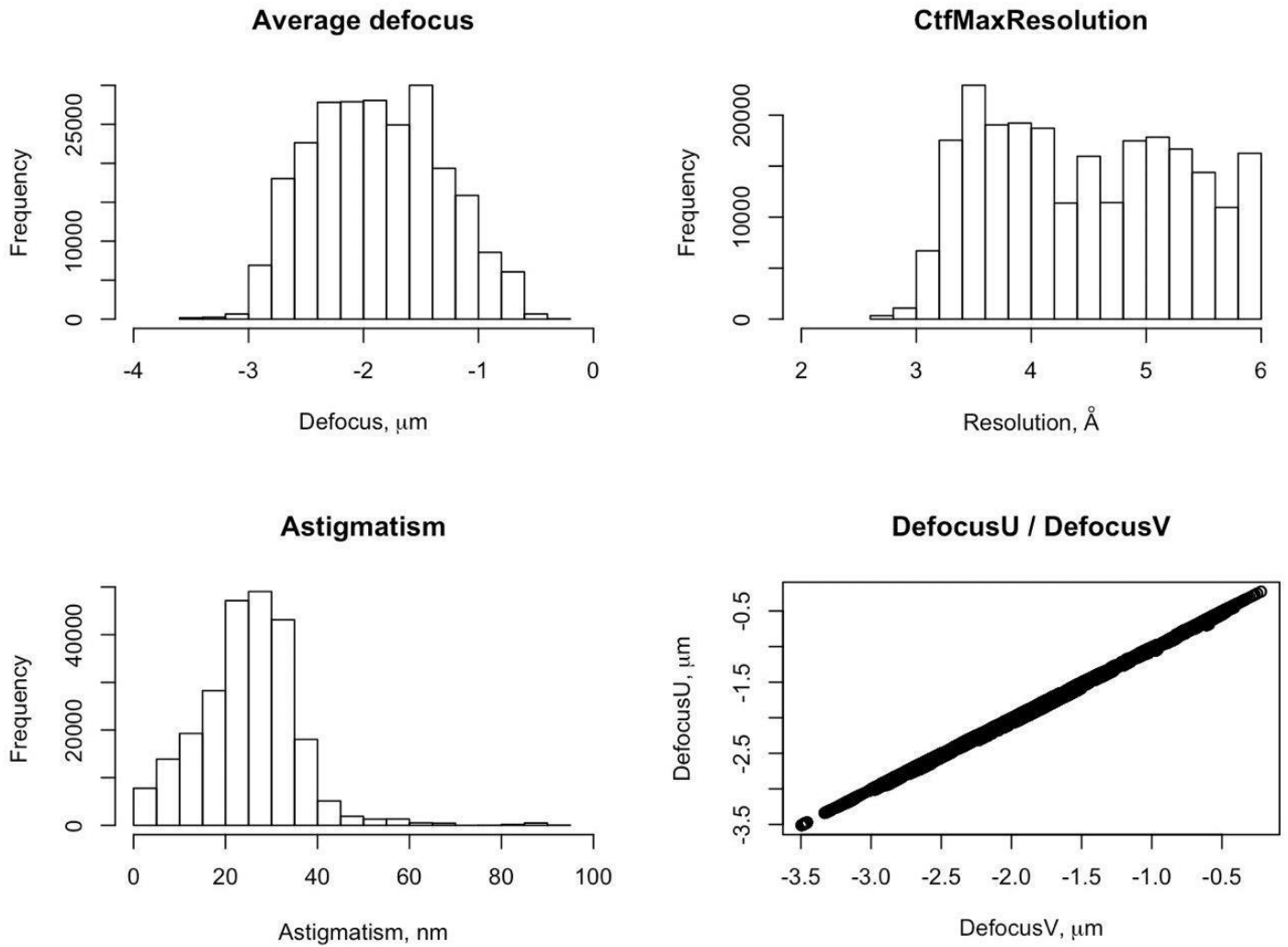


Figure 3. Class-averages of last stages of the 2D classification.

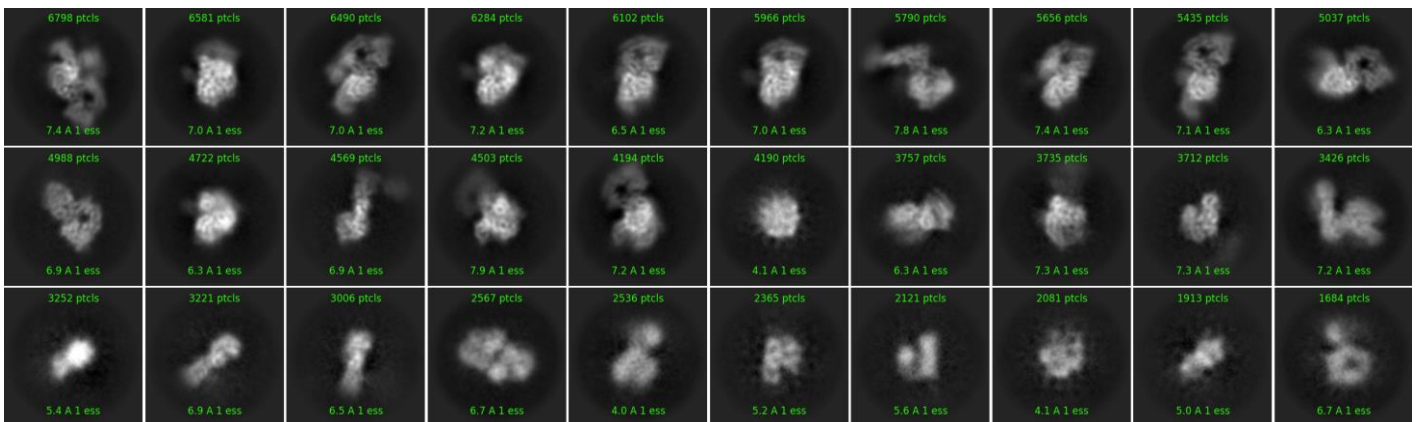


Figure 4. Final electron density for the helicase-primase complex. Top - two views rotated by 90° . Bottom - orientation distribution.

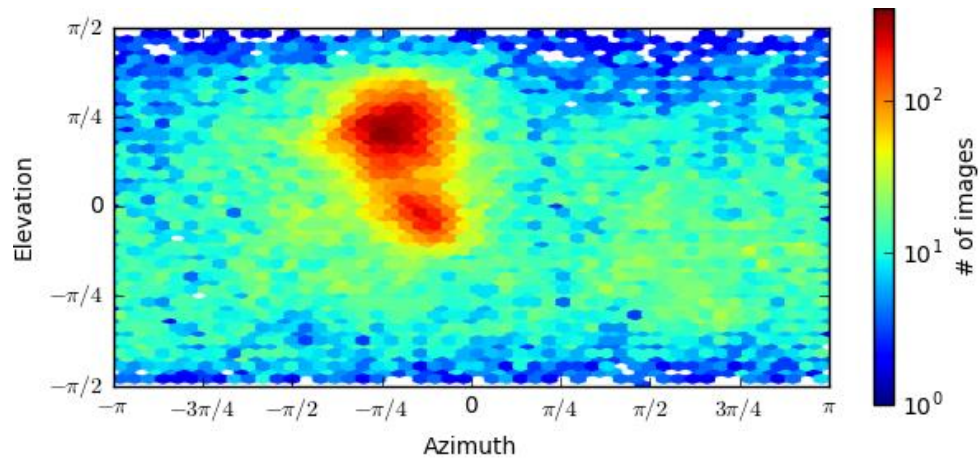
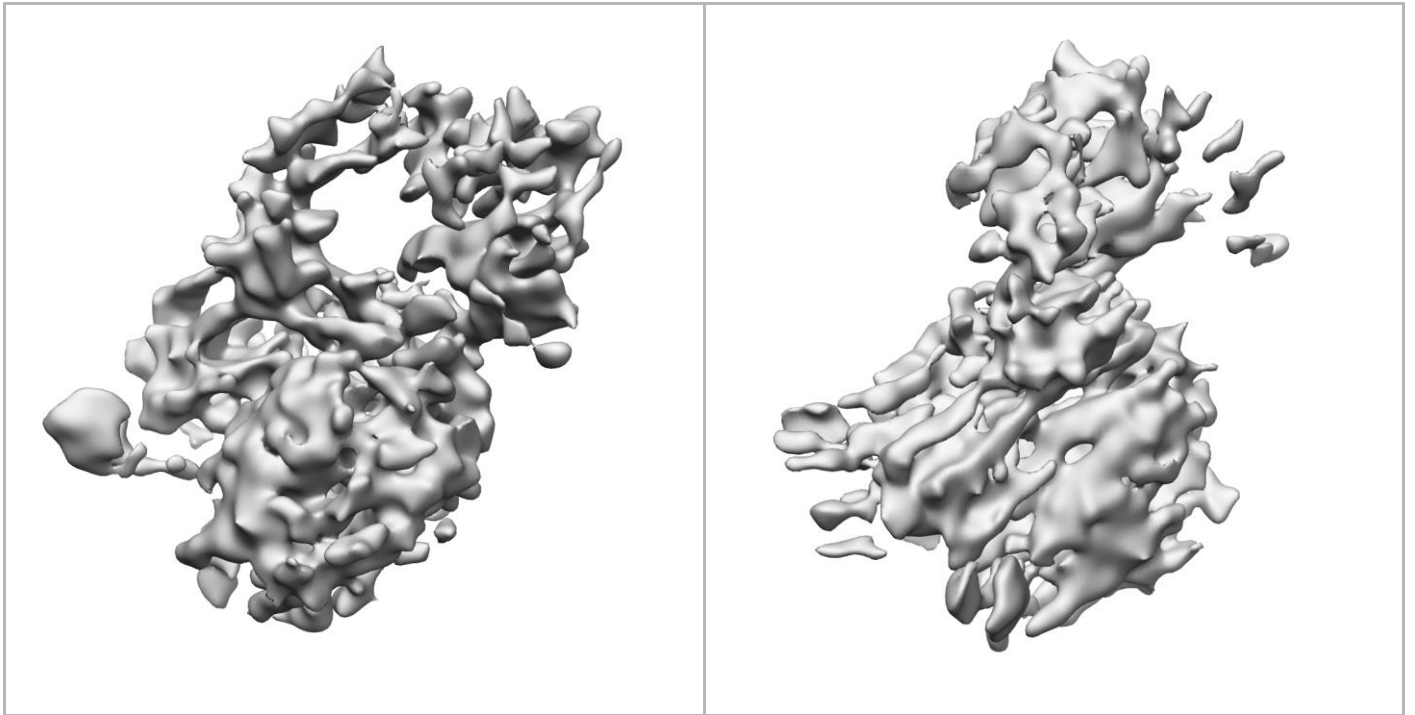


Figure 5. FSC curves and local resolution analysis.

