Approaches for Coherent X-ray Diffraction Imaging of *Paramecium bursaria Chlorella virus-1*

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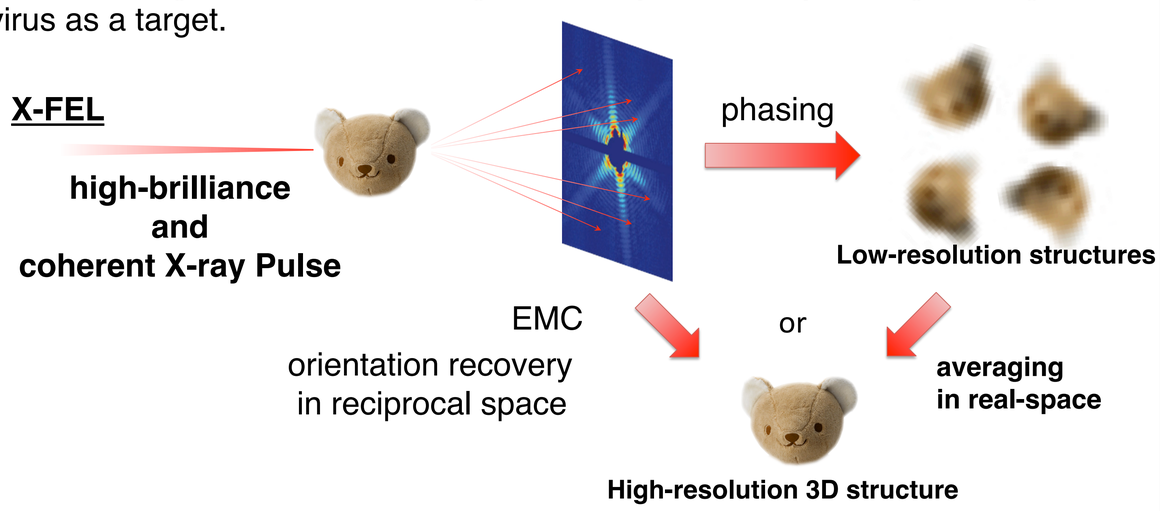
Coherent X-ray diffraction imaging

X-ray free electron lasers (XFELs) produce very short, extremely bright and coherent X-ray pulses. These features allow the imaging of single particles, without the need for crystallization, using a technique named coherent X-ray diffraction imaging (CXDI or CDI). The feasibility of CDI has been shown for a wide variety of particles ranging from small organelles (Hantke *et al.*, 2014), to large viruses (Seibert *et al.*, 2011), to small living cells (van der Schot *et al.*, 2015) in 2D, and recently for mimivirus in 3D as well (Ekeberg *et al.*, 2015). The resolution for 3D imaging however, is still limited. Our study will address several issues limiting the resolution, using a relatively structurally homogenous giant virus as a target.



Paramecium bursaria Chlorella virus-1 (PBCV-1)

PBCV-1 belongs to the genus *Chlorovirus* of the *Phycodnaviridae* family. Chloroviruses infect algal cells and exist in a freshwater environment throughout the world. PBCV-1 has a large linear dsDNAs (331-kbp) (Yamada *et al.*, 2006). The overall structure of the virus was determined by the cryo-electron microscopy and the outer capsid showed the symmetry of icosahedral T=169d. The inside of the outer capsid connected to a lipid bilayer membrane. The diameter of outer capsid is about 190 nm along the fivefold axis and 165 nm along the pseudo-two and pseudo-threefold axes (Yan *et al.*, 2000).

Preparation of PBCV-1 for CXDI experiments

Host : *Chlorella*

Infection at nearly OD(590 nm)=0.9

↓

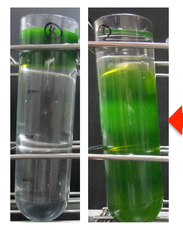
Purification after 72h of infection

↓

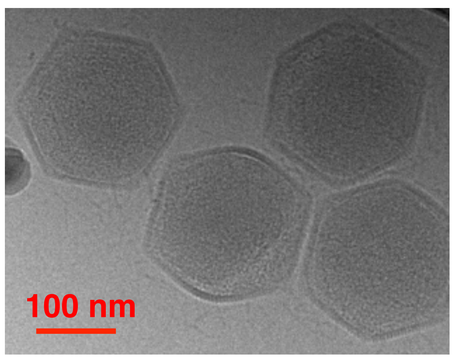
Sucrose density gradient centrifugation

↓

Filtering (0.4 mm)

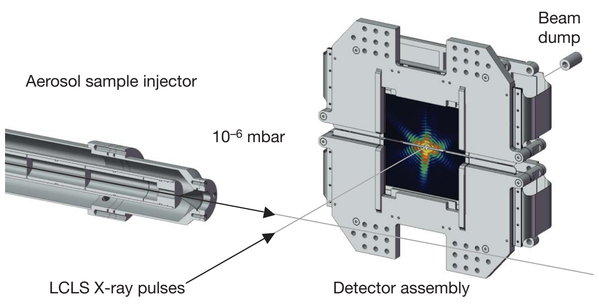


PBCV-1 / 1 GDa



Data collection

The CDI experiment was carried out at the Atomic, Molecular and Optics (AMO) end-station of the LCLS. Virus particles were introduced into the XFEL focus using an aerosol injector was used for suplying the virus to the XFEL, and the diffraction data set were collected. The diffraction patterns of over 200,000 hits were identified and processed with the *Cheetah* software package.

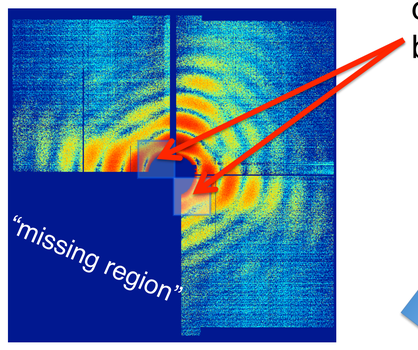


Seibert *et al.*, 2011

Run#	Time (H:M:S)	# of hit images
1	0:01:12	background
2	0:13:49	36,452
3	0:08:30	21,822
4	0:03:30	7,978
5	0:10:22	19,800
6	0:06:01	11,834
7	0:07:25	14,898
8	0:02:52	5,739
9	0:00:32	520
10	0:08:15	18,325
11	0:04:57	21,920
12	0:19:25	50,552
Total	1:25:38	209,840

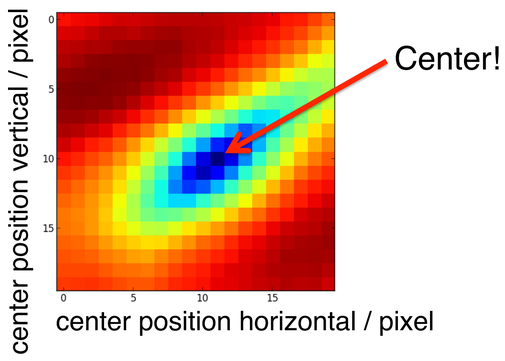
Finding center of images & recovering missing regions

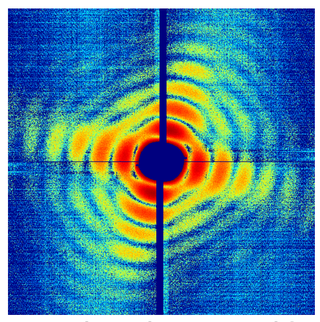
The quadrant of collected images were noisy and a poor quality. To recover missing or low quality regions, the center of diffractions were determined using by Friedel's law.



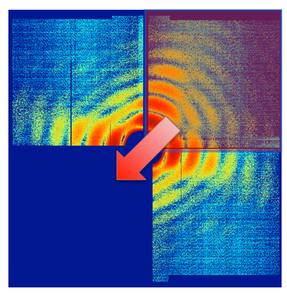
masked image

calculating the correlation between two regions based on the hypothetical center





recoverd and cropped image

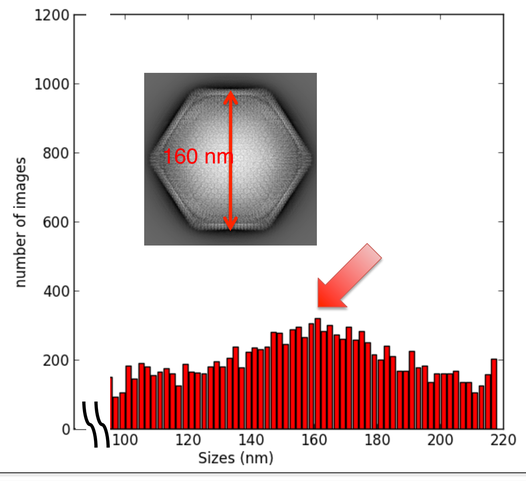


Correct center can recover the missing regions

Cropping at precise center

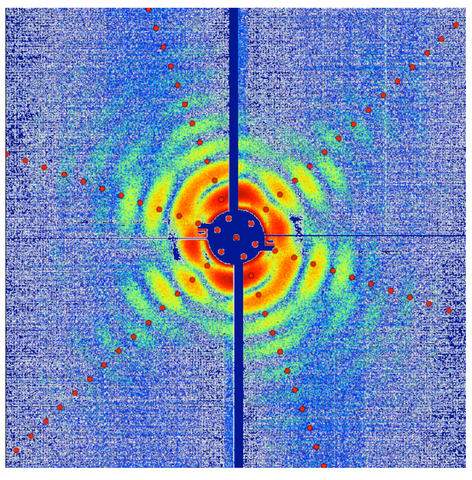
Sizing of particles from diffraction images

The size of PBCV-1 was calculated from autocorrelation functions. The size of PBCV-1 from cryo-EM map is ca.160nm. The size distribution of collected images roughly fitted to the suggested size.



Streak finding

To support for the orientation recovering in reciprocal space, the direction of streaking patterns were estimated from diffraction images.



Phase retrieval

Phases were retrieved with the Hawk software (Maia *et al.*, 2010). Reconstructions were carried out starting from random initial phases. These reconstructions consisted of 2,000 iterations with the RAAR algorithm, using a Shrinkwrap algorithm for support determination, and concluded with 2,000 iterations by the ER algorithm. Resolution for each reconstructions was estimated from the Phase retrieval transfer function (PRTF). Each reconstruction was iterated 100 times with independent and random starting phases.