: Electron microscopy on phages

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The aim of this practical is to prepare a phage sample for electron microscopy. Phages are viruses that infect bacteria. They can be large (jumbo phage) with an icosahedral head and contractile tail. The only way to image this kind of virus is electron microscopy.

There will be five parts in the practical:

Sample preparation by negative staining. This technique is fast (10 min) and quite easy. All the students will be able to practice and prepare their own grid. It will show how to prepare and screen the quality of a sample by electron microscopy.

Observation of the prepared EM grids in a low-grade electron microscope (LaB6 T12) and data acquisition on a CCD camera. The students will collect their own data on their own grid.

A cryo-EM (frozen hydrated technique, second kind of sample preparation for electron microscopy, Nobel Prize in Chemistry 2017), grid will be prepared using a Vitrobot. The advantages and disadvantages of this technique will be explained.

The cryo-EM grid will be loaded first in a Glacios electron microscope equipped with a last generation direct electron detector (IBS). Some images and movies will be acquired. Advantages and disadvantages of direct electron detector compared with CCD camera will also be discussed.

Finally, we will visit the ESRF EM platform equipped with a state-of-the art Krios electron microscope. Explanations on how to launch an automatic acquisition session will be given. If an automatic acquisition session is running, the students will get explanation on the different steps in real time.

Some ideas about image analysis and 3D reconstruction will also be given.
The practical will start at the IBS and continue at ESRF.

Titan Krios at ESRF