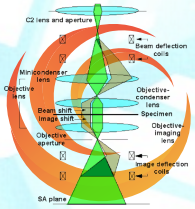
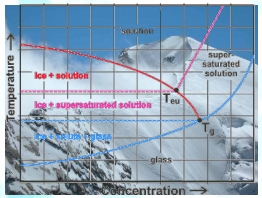


Cryo-Electron Microscopy Facility

PI: Terje Dokland, Ph.D.

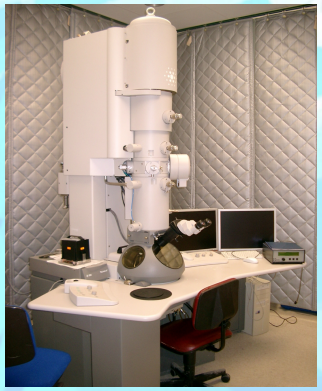
Shelby B40 (Basement) 1825 University Blvd.

Part of the Center for Structural Biology



High Resolution Cryo-EM

The Cryo-Electron Microscopy Facility provides capabilities for high resolution electron microscopy and tomography of stained and unstained specimens. Cryo-EM allows the observation of biological samples in their native environment, in the absence of the distortions and artifacts associated with traditional sample preparation methods, and is suitable for proteins and protein complexes, viruses, fibers, liposomes and intact prokaryotic cells up to about 1µm thickness. The instrument are available for use by trained users (training is available) or as a core service.

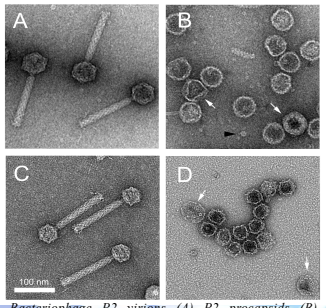


Tecnai F20 electron microscope features:

- 200 kV acceleration voltage
- Field-emission gun (FEG) electron source
- Gatan Ultrascan 4000 4k x 4k pixel high-sensitivity CCD camera
- Gatan 626 high-tilt cryo-sample holder, tilt range up to ±70°
- Tomographic data acquisition software
- Low dose Imaging mode
- FEI Vitrobot for sample preparation

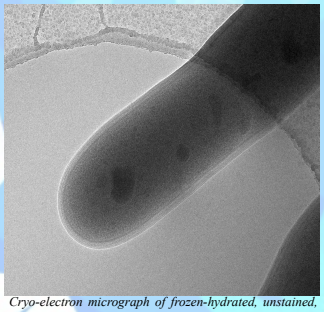
The instrument is available for use by trained users (training is available, contact Dr. Terje Dokland) and as a core service (contact Cindy Rodenburg for scheduling)

Negative stain



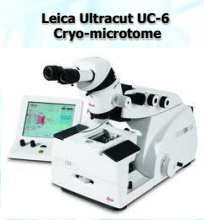
Bacteriophage P2 virions (A) P2 procapsids (B) bacteriophage P4 virions (C) P4 procapsids, negatively stained with 1% uranyl acetate and imaged at 50,000X with the F20 microscope. (Chang et al. 2008 Virology 370, 352-361) (UAB)

Cryo-EM



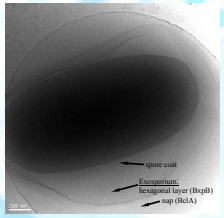
Cryo-electron micrograph of frozen-hydrated, unstained, intact Mycobacterium tuberculosis cell imaged at 29,000X. (Cells provided by Dr. Adrie Steyn, UAB)

Cryo-sectioning

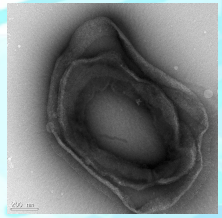


A Leica UC6 microtome is available for cryo-sectioning of frozen bulk samples, such as cells, organelles and tissues. Sections can be processed by the Tokuyasu method and used for antibody labeling.

(Traditional plastic sectioning is available in the HRIF EM core.)



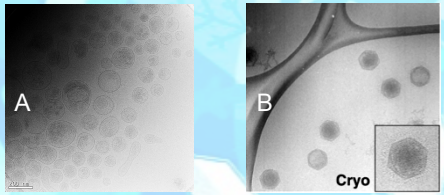
Bacillus anthracis (Sterne) spore, imaged by cryo-EM. The hexagonal basal layer and fibrous nap of the exosporium are clearly visible.



100 nm cryo-section of B. anthracis spore, prepared by the Tokuyasu method and stained with 1% uranyl acetate, revealing the multiple ordered layers in the spore coat and exosporium. (B. anthracis spores were supplied by C.L.Turnborugh, UAB)

Lipid-containing samples

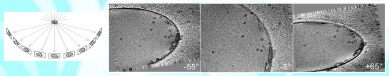
Cryo-EM is ideal for imaging lipid-containing samples, such as lvesicles and enveloped viruses that are poorly preserved by traditional methods.



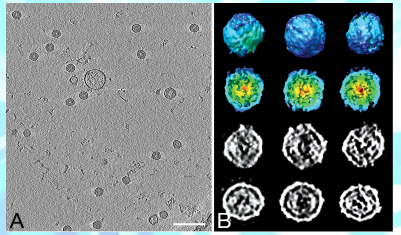
(A) Exosomes from human semen (prostasomes) imaged by cryo-EM. The integrity, shape and multilayered appearance of the prostasomes are preserved (Poliakov et al. 2009 Prostate 69, 159-167). (B) Wiseana iridescent virus (WIV), a large insect virus, imaged by cryo-EM. The fibers and internal lipid membrane are clearly discernible. (Juhl et al 2006 Adv Funct Mat 16, 1086-1094)

Electron Tomography

Tomography is a completely general reconstruction procedure that can be applied to individually unique, non-symmetrical samples, such as lipid vesicles, enveloped viruses and whole cells. In electron tomography, images of the sample are taken at multiple angles (±70°) with extremely low electron dose. The images are combined computationally by backprojection to a 3D reconstruction.



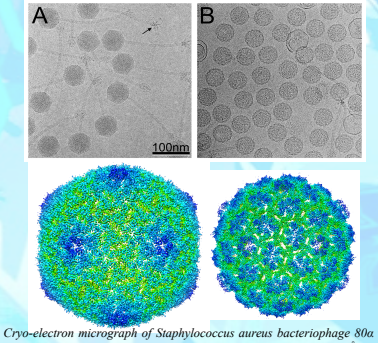
Images of porcine reproductive and respiratory virus (PRRSV) at three different tilt angles.



(A) Central section (15Å thick) through the resulting PRRSV tomogram. In (B), three individual virus particles from (A) were segmented out of the tomogram and shown in isosurface representation (top two rows) and in section (bottom rows). (Spilman et al. 2009 J. Gen. Virol. 90, 527-535)

3D Reconstruction

Cryo-EM allows high resolution 3D reconstruction by combining information from multiple identical particles in different orientations, such as viruses, ribosomes etc.



Cryo-electron micrograph of Staphylococcus aureus bacteriophage 80α virions (A) and procapsids (B). The corresponding 3D reconstructions at 9Å resolution are shown below the micrographs. (Spilman et al. 2011. J. Mol. Biol. 405, 863-876)

For queries and training, contact:
Terje Dokland, Ph.D.
Core Director
996-4502 (Office)
dokland@uab.edu

For usage and scheduling, contact:
Cindy Rodenburg
Electron Microscopy Specialist
996-4510 (Office)
cindyr@uab.edu