O'NEAL COMPREHENSIVE CANCER CENTER
THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
UAB Institutional Research Core Program

Cryo-Electron Microscopy Facility

Director: Terje Dokland, Ph.D. Facility Manager: James Kizziah, Ph.D.

NIH National Institutes of Health
Turning Discovery Into Health
S10 OD024978 (Dokland)
P30 CA013148 (Sleckman)

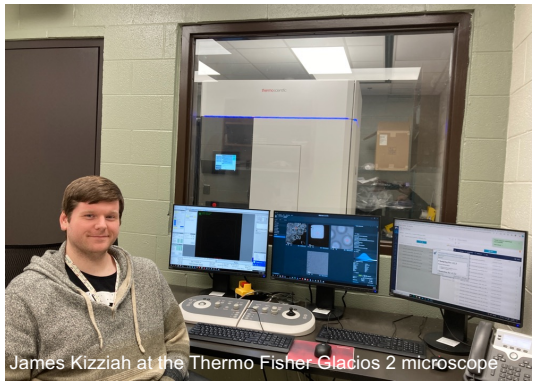
Equipment and capabilities

The UAB Cryo-Electron Microscopy Facility (CEMF) 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. Cryo-EM only requires 3 µl of sample per grid at a concentration of ≈ 1 µM. Many samples that are too flexible or heterogeneous for other structural biology approaches may be amenable to cryo-EM. In combination with three-dimensional reconstruction procedures, cryo-EM is capable of determining near-atomic resolution (<4Å) structures of proteins from ≈100kDa to multi-MDa macromolecular complexes. Additionally, electron tomography can be used to generate 3D structures of pleomorphic objects like cells and organelles.

CEMF equipment includes:

Thermo Fisher Glacios 2 electron microscope
- 200kV FEG with 12-grid Autoloader
- Falcon 4i direct electron detector
- Ceta-16M CMOS detector
- EPU Multigrid and EPU Tomo software
- Data throughput 600 images/hr
- Attainable resolution ≤2.2 Å

Talos F200C electron microscope
- 200kV FEG, Gatan 626 and 698 side-entry specimen holders
- Direct Electron Apollo detector
- Ceta-F CMOS detector
- EPU and SerialEM software


James Kizziah at the Thermo Fisher Glacios 2 microscope

Other equipment:
- FEI Vitrobot Mark IV
- Gatan CryoPlunge
- Pellco easiGlow glow discharger
- Tergeo-EM plasma cleaner
- Leica UC7 cryo-microtome (available through HRIF)

Services offered

- Sample preparation for negative stain and cryo-EM
- Room temperature and cryogenic imaging and data collection
- Computational services: data processing, 3D reconstruction and analysis
- User training in sample preparation, imaging and data processing methods

- **Rates (internal users§):**

Microscope usage, room temperature:	$55 / hr
Microscope usage, cryogenic*:	$60 / hr
Cryo-EM sample preparation:	$50 / session
Operator assistance/labor:	$60 / hr
Computational services:	$60 / hr
Grids:	$6–$20 / each

*Rates for Glacios data collection are currently capped at $480 + labor per 24-hour period per user
§External academic users pay 50% more; non-academic users pay 200% more.

Negative stain imaging

Negative staining and room temperature imaging can be used for a wide variety of samples and is useful for assessing sample quality and concentration. In combination with immuno-gold or Ni-NTA-gold labeling, negative stain images can identify locations of specific proteins in the sample.

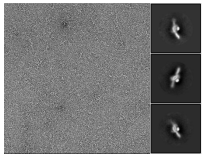
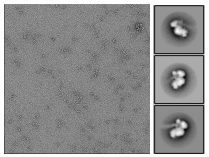
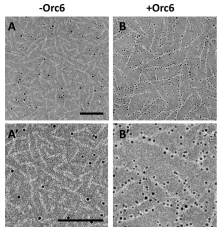


Image of negatively stained GBF1, a member of the Sec7 family of ARF-activating guanine exchange factors. Right, representative 2D class averages. Elizabeth Sztul, UAB.

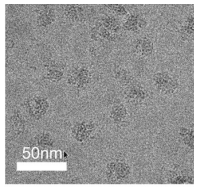

Negatively stained Streptococcus mutans glucosyl transferase GtfD. Right, 2D class averages. Champion Deivanayagam, UAB.



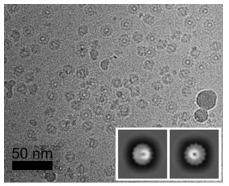
Septin filaments in the presence of His6-Orc6, labeled with Ni-NTA-gold. Akhmetova et al 2015 Mol. Biol. Cell 26. (Igor Chesnokov, UAB).

Cryo-EM imaging

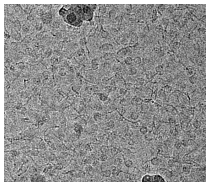
Cryo-EM can be used for imaging proteins and protein complexes as small as 100-150 kDa, as well as ribosomes, viruses and small cells up to ≈1 µm thickness.

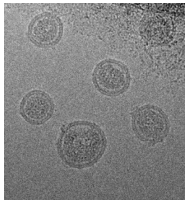


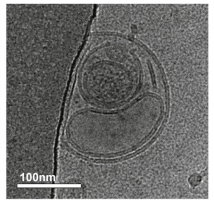
Streptococcus pneumoniae ribosomes. Terje Dokland, UAB.



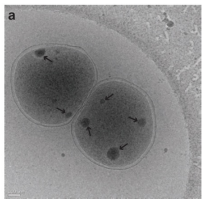
Portal proteins , a 720 kDa dodecamer from Staphylococcus aureus bacteriophage 80α. Inset, representative 2D class averages. Terje Dokland, UAB


Native human H2Aub nucleosomes. Hengbin Wang, Virginia Commonwealth University


Porcine reproductive and respiratory syndrome virus (PRRSV), showing the internal core. Spilman et al 2009 J Gen Virol 90, 527-535. Terje Dokland, UAB.



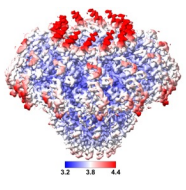
Human lung exosomes. Hough et al 2018 Redoc Biol. 18, 54-64. Jessy Deshane, UAB.



Prochlorococcus, an oceanic cyanobacterium. Arrows point to carboxysomes. Hennon et al 2017 ISME J 2017.189. Jeff Morris, UAB.

3D Reconstruction

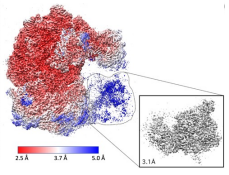
Cryo-EM can be combined with single-particle or tomographic reconstruction procedures to generate 3D structures of proteins, protein complexes, viruses and other specimens.



80α portal protein complex, 3.1 Å resolution.

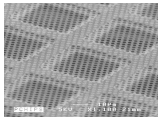

Phage Andhra (composite)

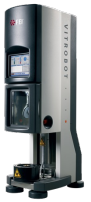

Sub-tomogram averaging of phage tails.

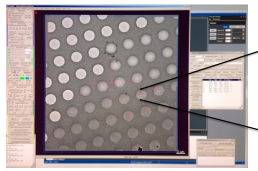
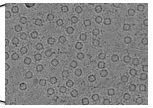


S. pneumoniae ribosome, 2.9 Å resolution.

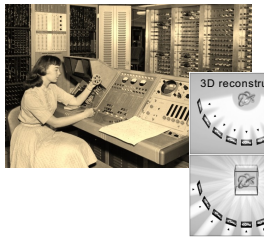
Cryo-EM Procedure

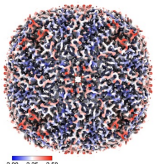


The sample (3 µl) is placed on a special perforated "holey film" support. Samples should be at 1 µM concentration in low ionic strength (<250 mM) solution…


Samples are frozen with the FEI Vitrobot…



Apoferritin

… and imaged in the microscope at –180°C using EPU or the SerialEM interface, here showing an image of the holey film with sample covering many of the holes. Images can be collected automatically with low electron dose at each hole.



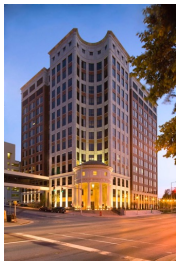


Apoferritin reconstruction at 2.2 Å resolution from Glacios 2 microscope

The image data are processed in GPU-accelerated workstations or on the Cheaha cluster to produce 3D representation of the structure. Depending on symmetry and size, from 10,000 to 500,000 particle images may be needed for an atomic resolution structure.

Location and Contact Information

We are located in Shelby B40, 1825 Univ. Blvd.
For scheduling, consultation and more information, contact:



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