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

UAB Cryo-Electron Microscopy Facility

NIH National Institutes of Health
Turning Discovery Into Health

S10 OD024978 (Dokland)
P30 CA013148 (Sleckman)

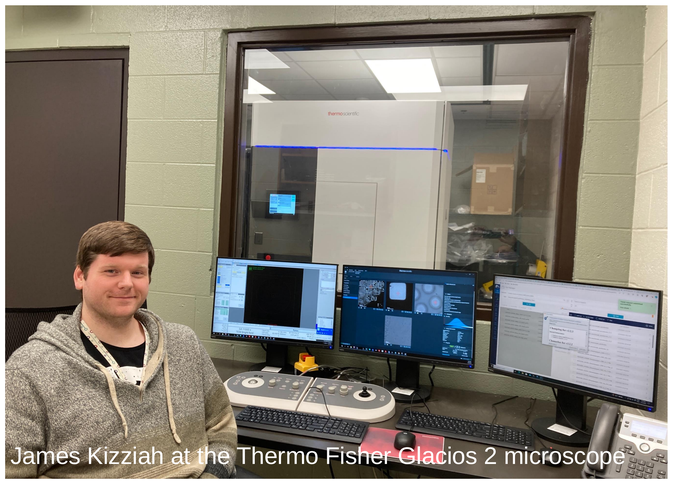
Equipment and capabilities

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
- 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 Multigrid and EPU Tomo software


James Kizziah at the Thermo Fisher Glacios 2 microscope

Other equipment:

- FEI Vitrobot Mark IV
- Pelco easiGlow glow discharger
- Tergeo-EM plasma cleaner
- 4-GPU data processing workstation
- 420TB data server

Contact UAB Research Computing for 25TB physical + 75TB cloud storage free for UAB labs.

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 / hr
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.

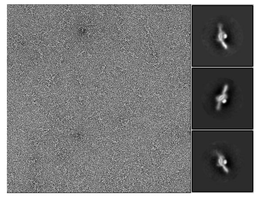
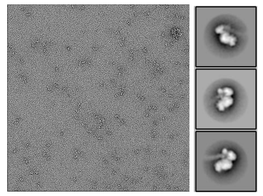
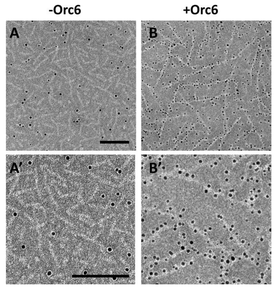


Image of negatively stained GBF1, a member of the Sec7 family of ARF-activating guanine exchange factors. Right, representative 2D class averages. Meissner et al 2023. Front. Cell. Dev. Biol. 11, 1233272. (Elizabeth Sztul, UAB)

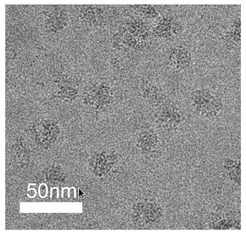

Negatively stained Streptococcus mutans *glucosyl transferase GtfD. Right, 2D class averages. Champion Deivanayagam, UAB.*



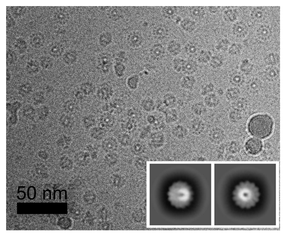
Septin filaments in the presence of His_6-Orc6, labeled with Ni-NTA-gold. Akhmetova et al 2015 Mol. Biol. Cell 26. (Igor Chesnokov, UAB).

Cryo-EM imaging

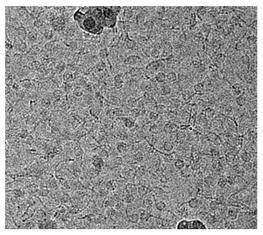
Cryo-EM can be used for imaging proteins and protein complexes as small as 100-150 kDa, as well as ribosomes, viruses and small cells up to ≈1 µm thickness.

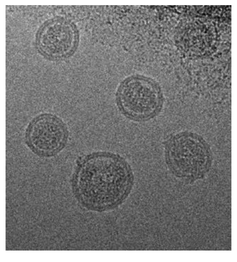


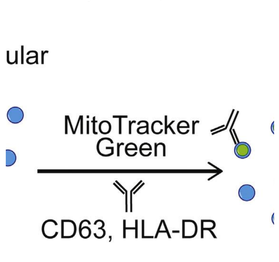
Streptococcus pneumoniae ribosomes. Terje Dokland, UAB.



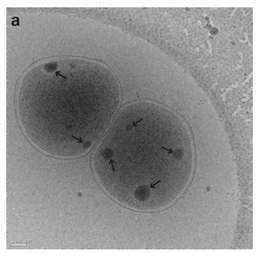
Bacteriophage 80a portal protein. Inset, representative 2D class averages. Mukherjee et al 2024 J. Mol. Biol. 436, 168415. Terje Dokland, UAB


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.



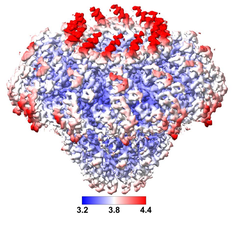
Human lung exosomes. Hough et al 2018 Redox Biol. 18, 54-64. Jessy Deshane, UAB.



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

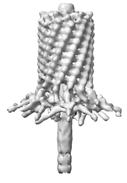
3D Reconstruction

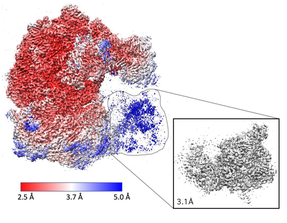
Cryo-EM can be combined with single-particle or tomographic reconstruction procedures to generate 3D structures of proteins, protein complexes, viruses and other specimens.



80a portal protein complex, 3.1 Å resolution.

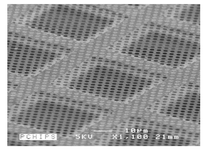

Phage Andhra (composite)


Sub-tomogram averaging of phage tails.

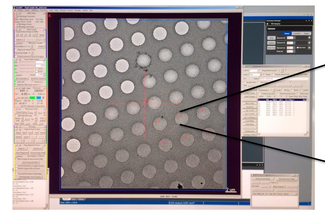
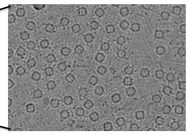


S. pneumoniae ribosome, 2.9 Å resolution.

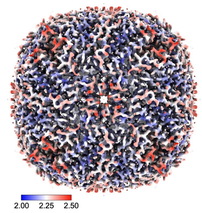
Cryo-EM Procedure



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 a 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

For scheduling, consultation and more information, visit out website:

The CEMF is located in Shelby B40, 1825 Univ. Blvd.

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