

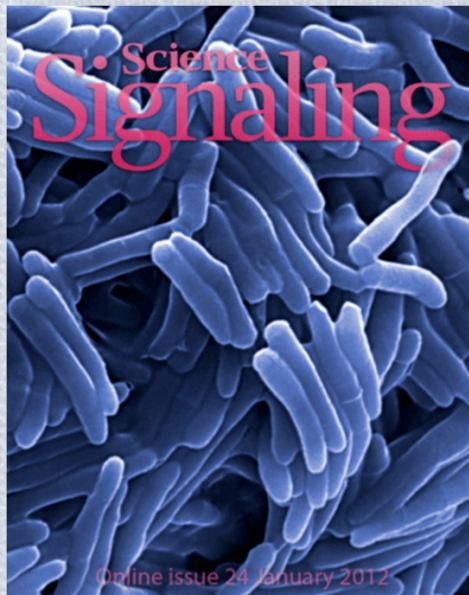


Core Electron Microscopy Facility

established 1998.

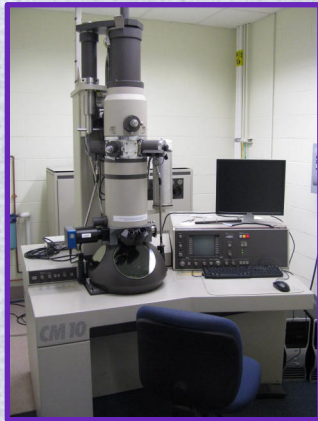
Staff: Greg Hendricks, Ph.D. (Manager)
Lara Strittmatter, Ph.D. (Assistant Manager)
Keith Reddig (Research Associate)

Co Directors: George Witman and Roger Craig



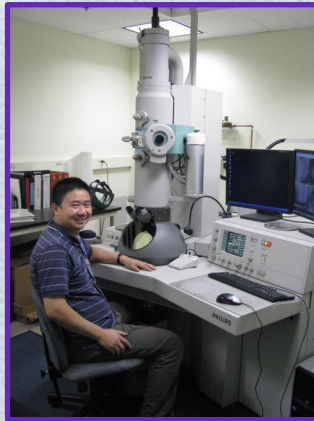
Instrumentation

Transmission Electron Microscopes (TEM)



Routine
RT
imaging

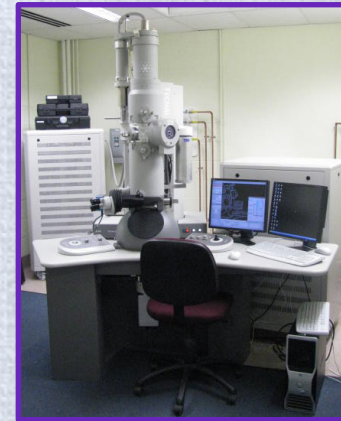
Phillips CM 10



Low dose
and cryo
imaging

New fast
scan digital
imaging
system

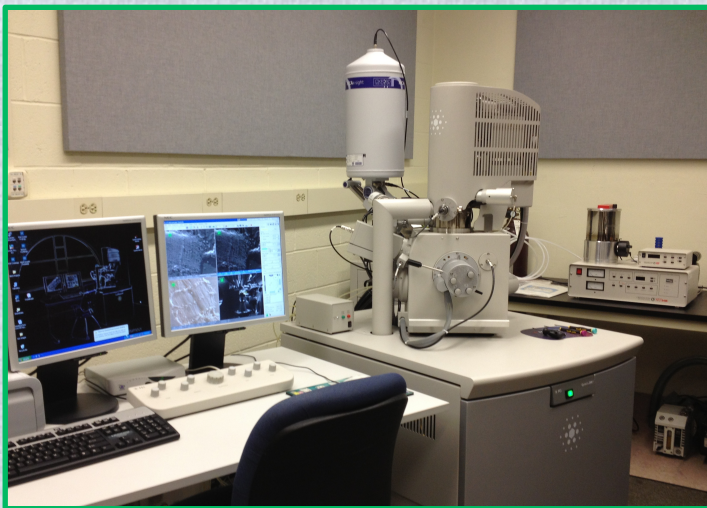
Phillips CM 120 Cryo



Routine RT
and cryo
imaging
with
tomographic
capabilities

FEI Tecnai Spirit 12

Scanning Electron Microscope (SEM)



High vacuum, variable pressure and environmental
modes (ESEM)

High resolution field emission gun (1.5 nm in high
vacuum mode)

Oxford Link Inca 350 x-ray spectrometer for light
element detection and x-ray spectral imaging.

FEI Quanta 200 FEG MKII

Services

Consultation and custom sample handling to meet the researchers needs.

A detailed floor plan of a laboratory facility. The plan shows various rooms including 'S.E.M. PREP. LAB RM #112', 'CORRIDOR RM #100CE', 'OFFICE RM #114', 'M.SCOPE RM #114A', and 'VET'. A red star is placed in a hallway area, and several black footprints are scattered across the plan, suggesting a path or activity. The text 'Consultation is free so please, come and talk to us!' is overlaid on the plan in a white box.

Consultation is free so please, come and talk to us!

The services include preparation of fresh samples to the final imaging stage or they can include/exclude any part of the process to accommodate the investigator.

Sample Preparation Work Flow

Specimen

Spread onto support grid

Negative Stain
or
Metal shadow

Chemical fixation

TEM

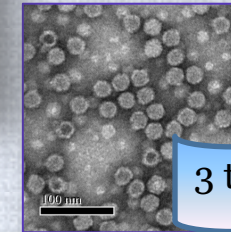
Dehydration

Embedding

2 to 3 weeks

SEM

Ultramicrotomy



3 to 5 days

Critical point drying

Immunolabeling

Immunolabeling

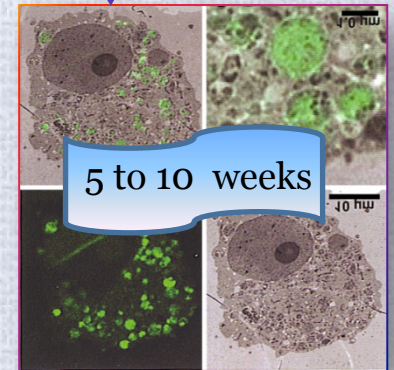
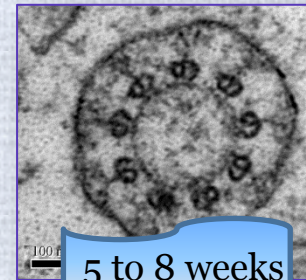
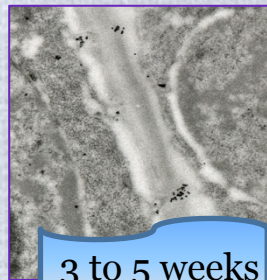
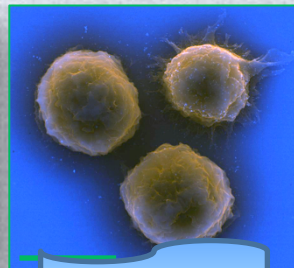
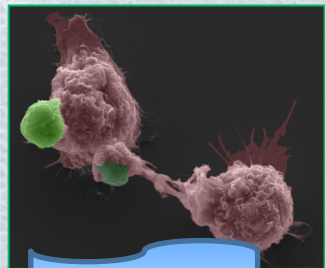
specific area (i.e. cilia in trachea or in eyes, islets in pancreas): thick sections stained with toluidine blue first

Correlative LM & TEM

Metal Coating

Carbon Coating

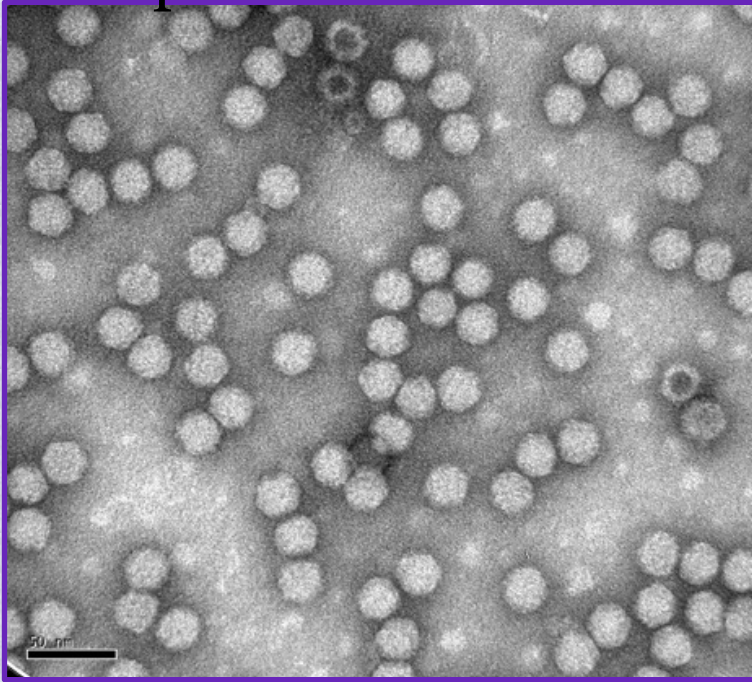
Contrasting



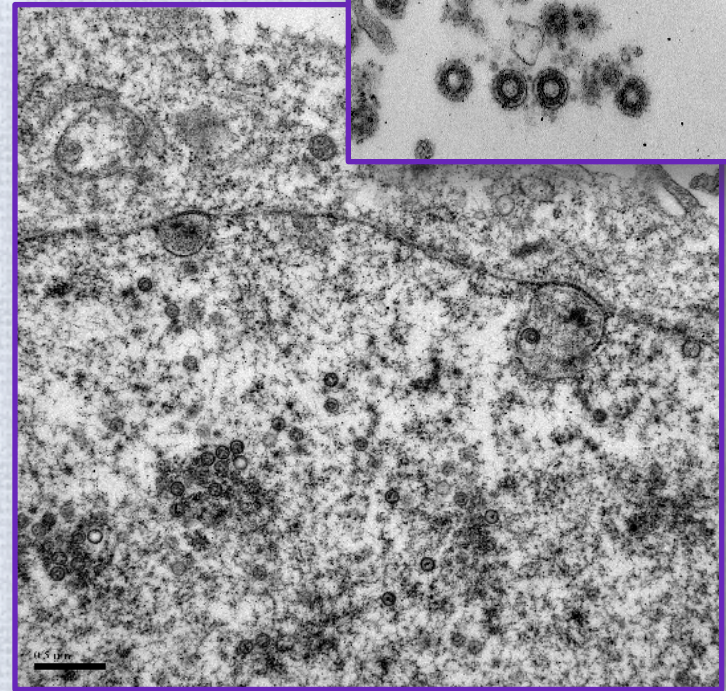
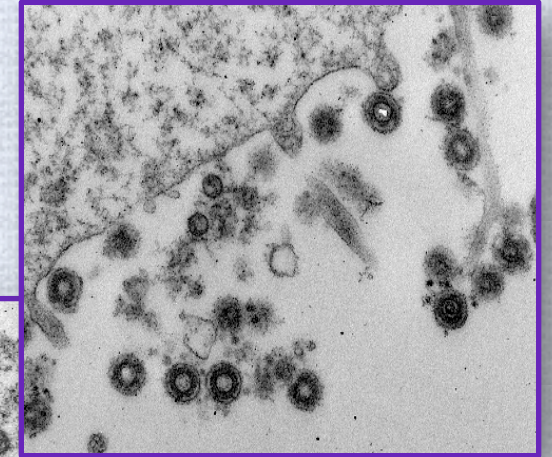
5 to 10 weeks

Examples

Viral particles

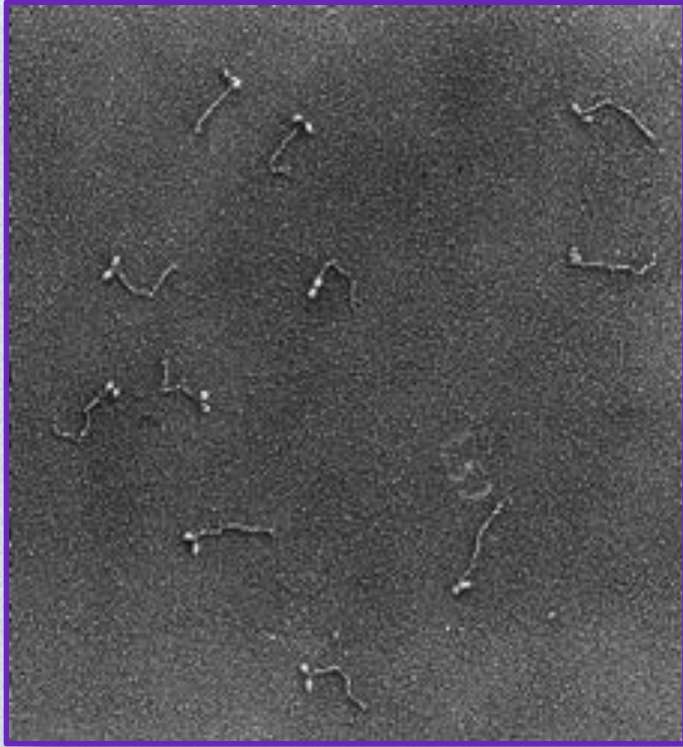


Negatively stained AAV



Embedded and sectioned
herpes virus

Examples



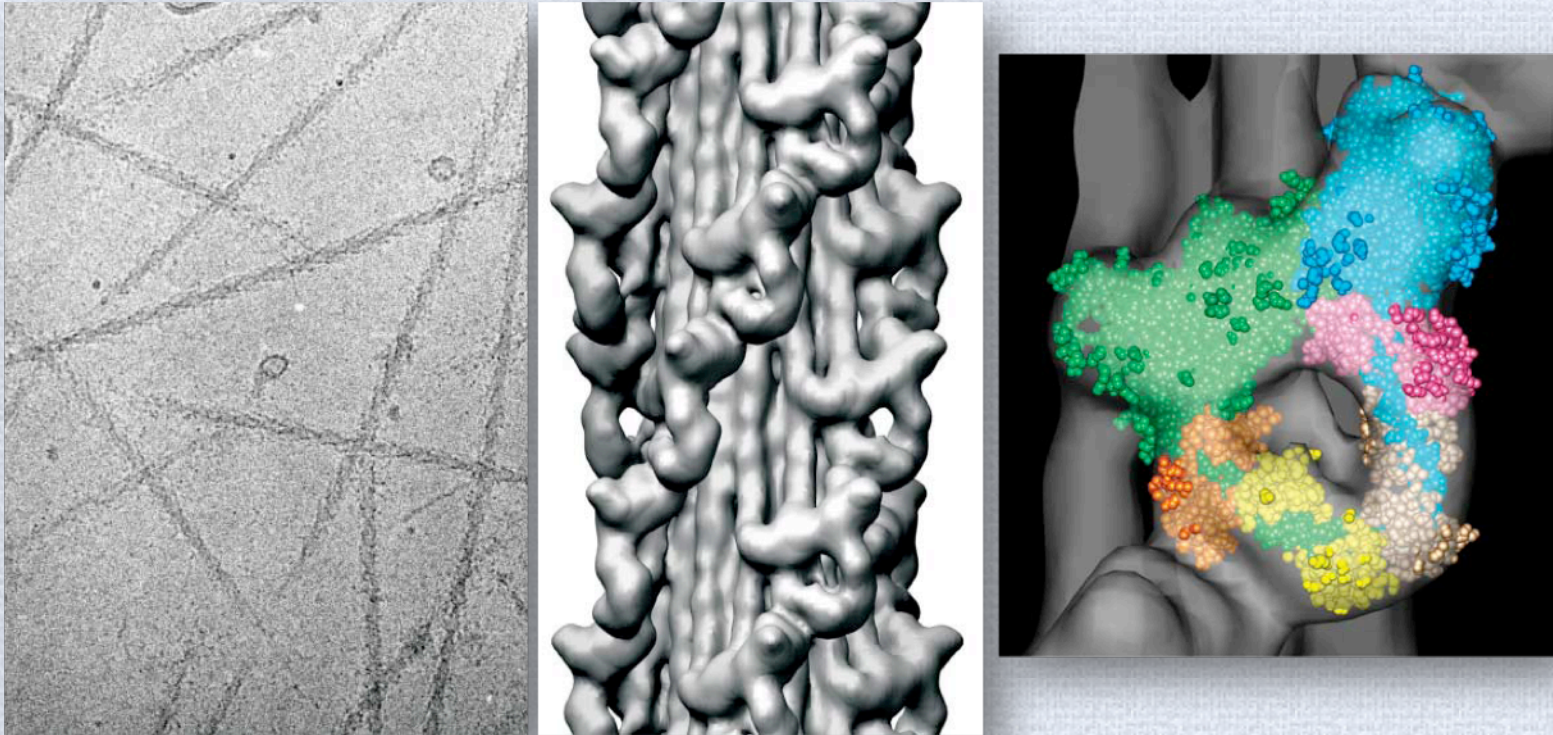
Rotary shadowed myosin molecules.
Photo: Craig Lab



Negatively stained decorated actin filaments. Photo: Craig Lab.

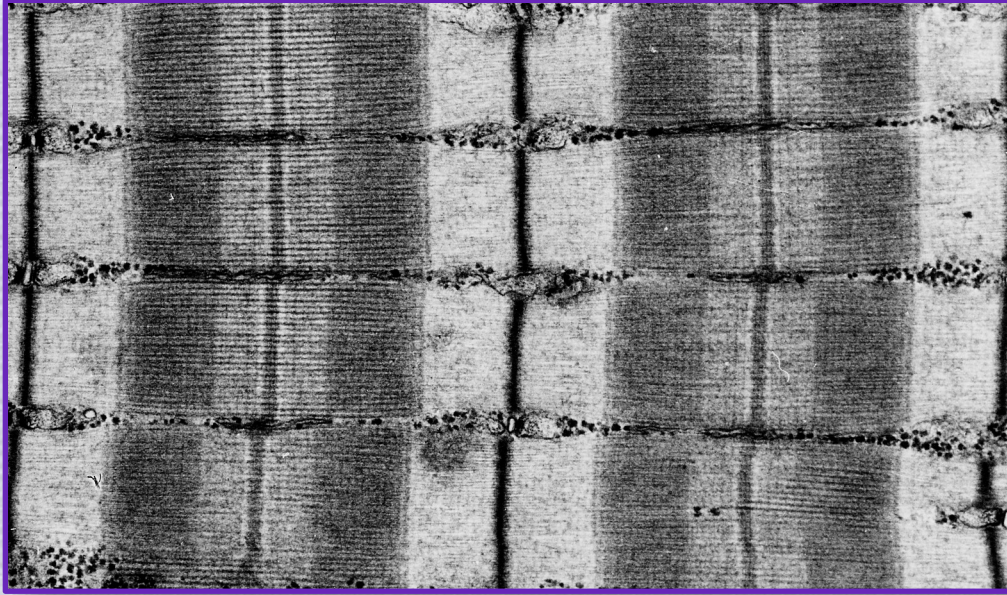
Examples

(Courtesy of Roger Craig)

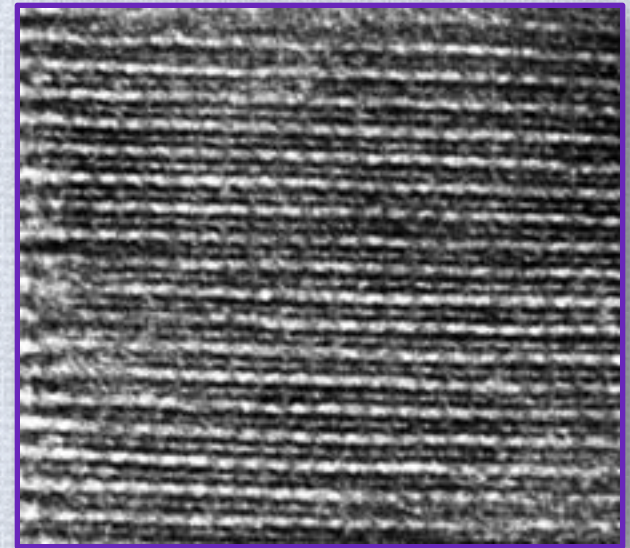


Cryo-electron micrograph and 3D single particle reconstruction of unstained, frozen-hydrated myosin filaments from muscle. Docking of myosin head crystallographic structure (right) into reconstruction leads to near atomic model of myosin filament (Craig lab; Woodhead et al., Nature 2005).

Examples



Thin sectioned epoxy embedded skeletal muscle

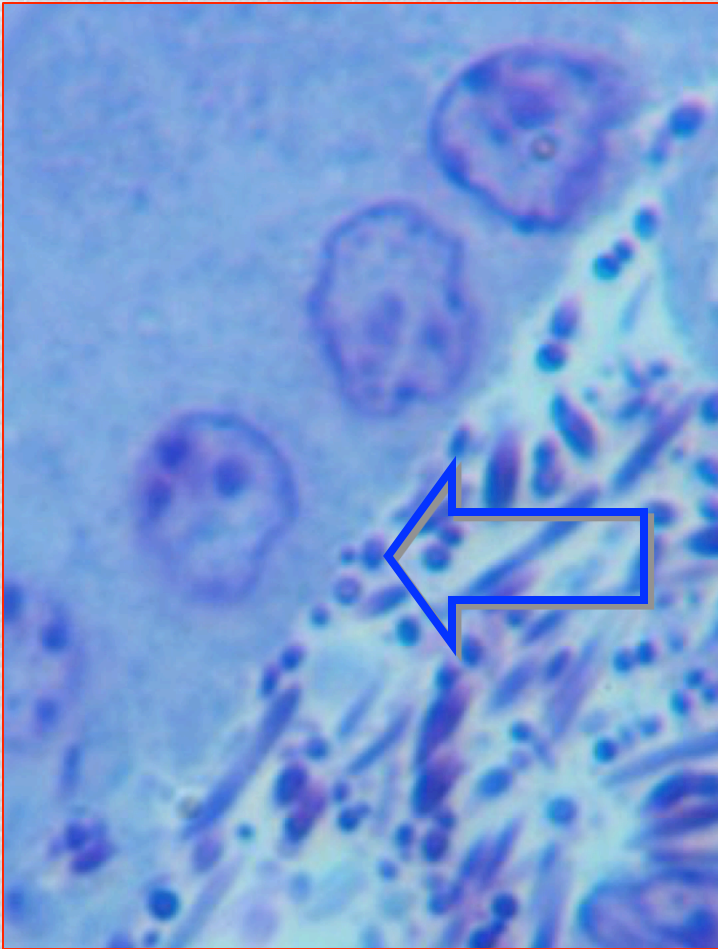


Negatively stained longitudinal cryo-section of muscle (myosin and actin filaments appear white). Photo: Craig Lab.

Examples

Light and TEM imaging

Bacteria on mouse intestines



Toluidine blue stained thick section

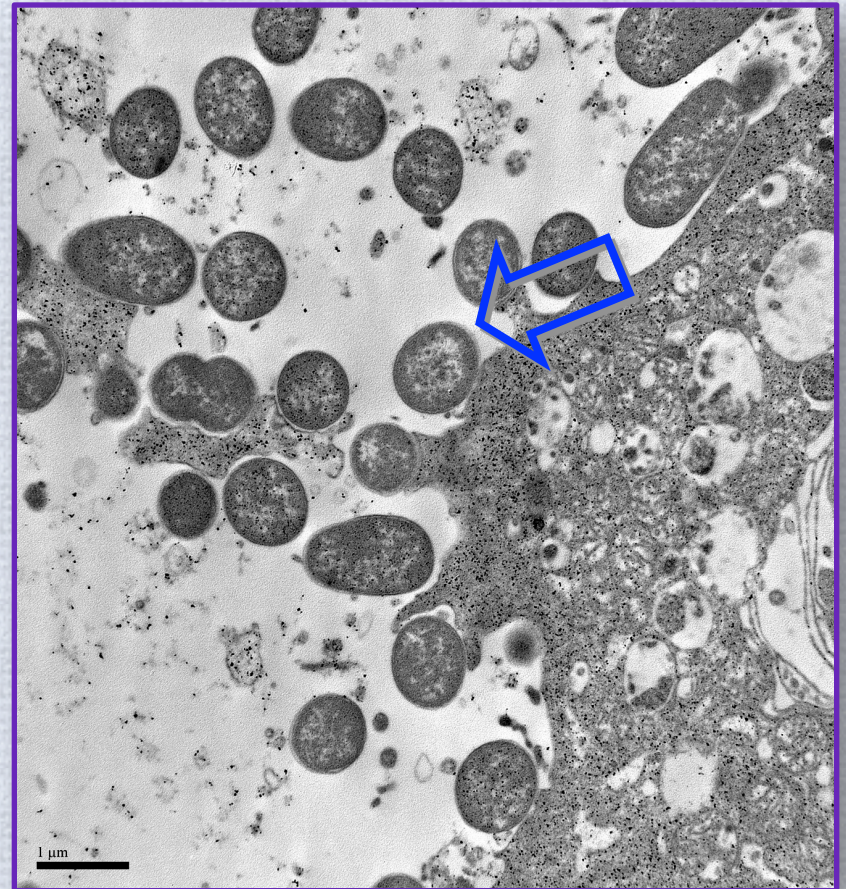
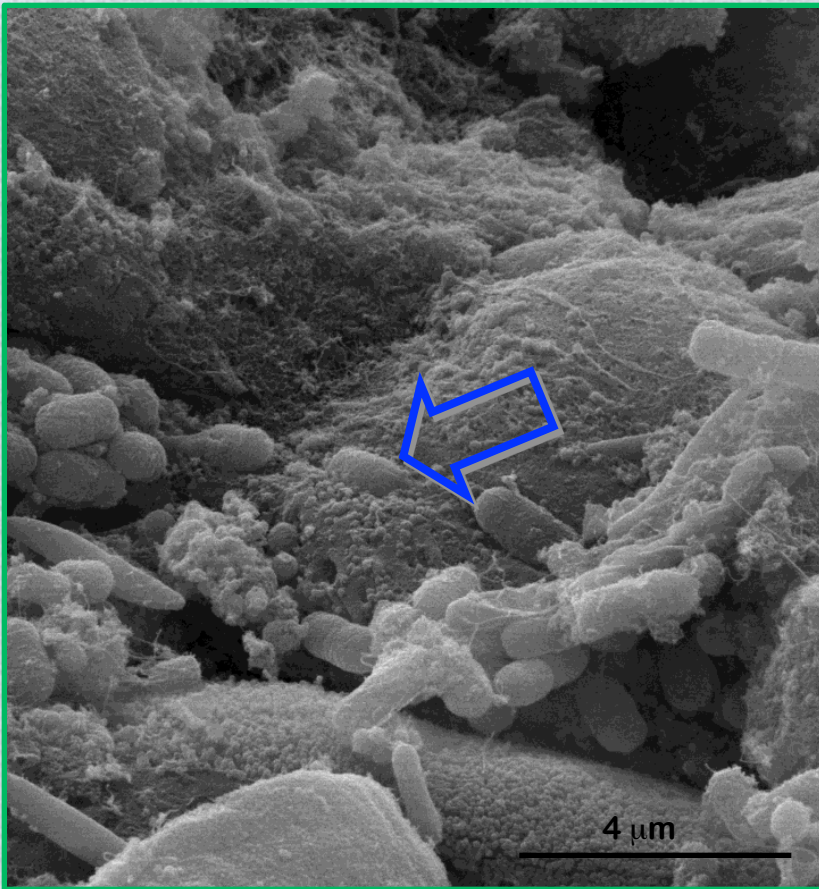


Ultrathin TEM section contrasted with lead citrate and uranyl acetate

Examples

SEM and TEM imaging

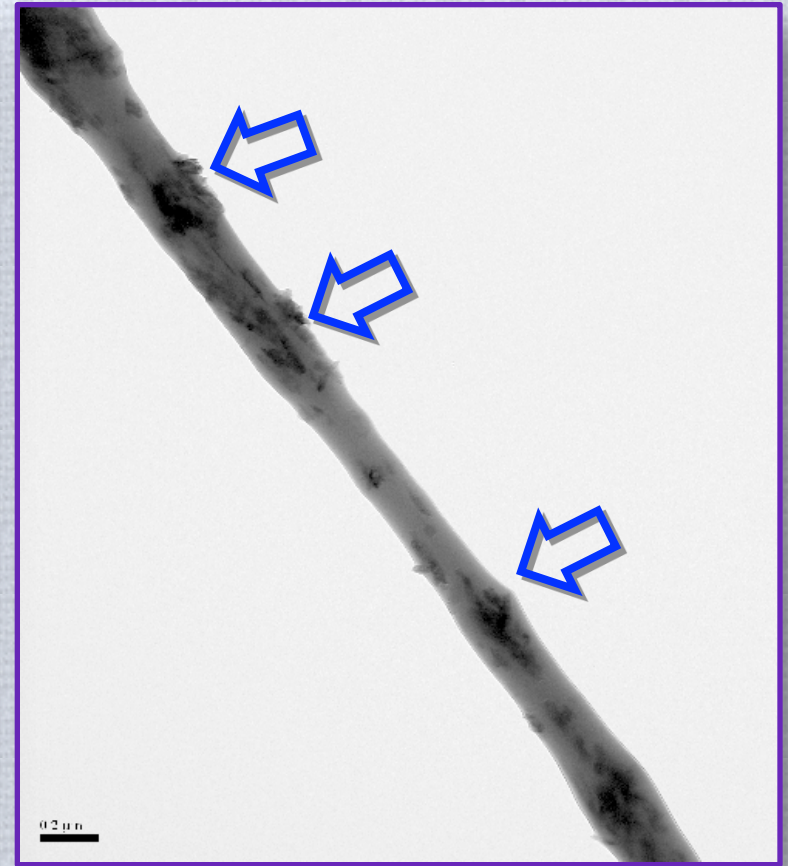
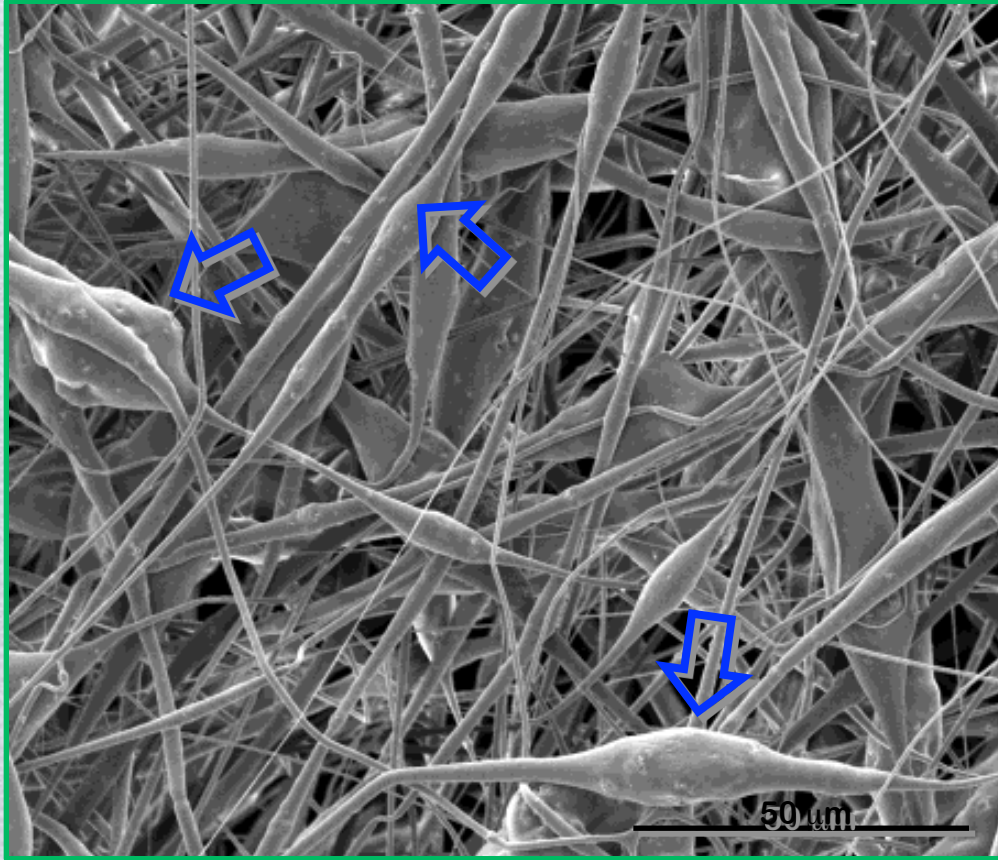
Bacteria on mouse intestines



Examples

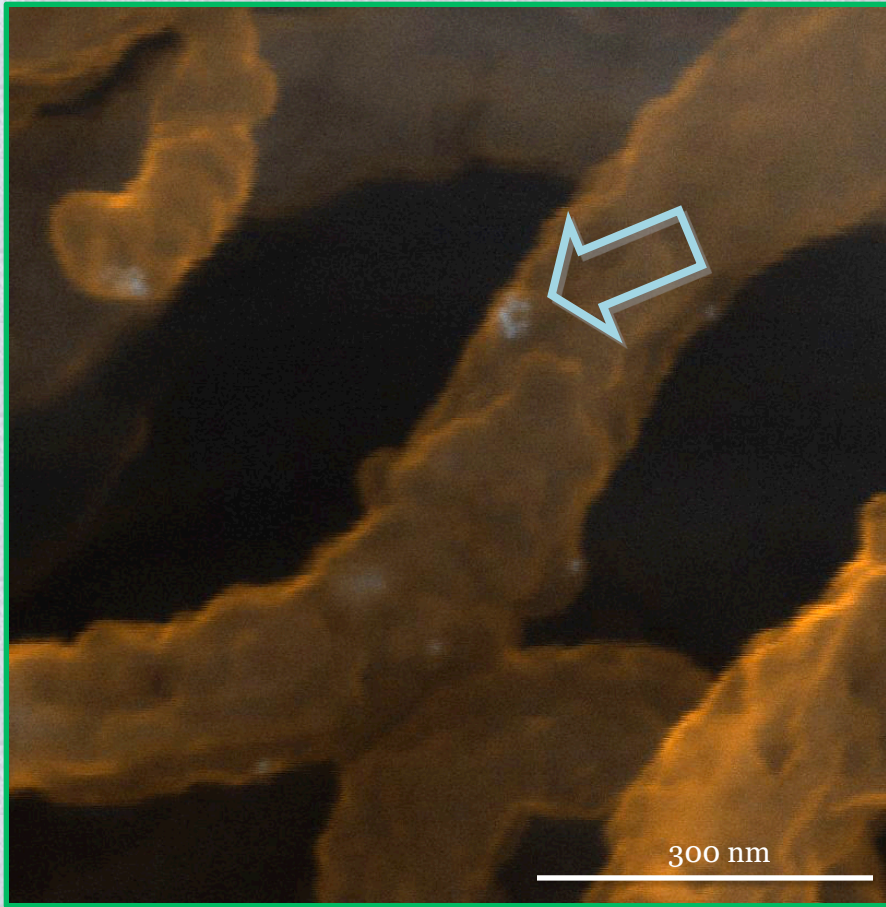
SEM and TEM imaging

Nanofibers with embedded crystals



Examples: Immuno Labeling

SEM and TEM imaging



Genetically modified spirochete with Lyme disease antigen



Autophagosome in HeLa cell

Summary

The EM Core Facility:

Provides UMMS investigators with advanced TEM (RT and Cryo) and SEM microscopy including:

- Consultation and Training
- Custom sample handling to meet the researchers needs
- Ancillary equipment for specimen preparation (including Cryo EM), and technical support

“Bye, and thanks for all the fish!”

