Cryo-preservation and Freeze-Substitution

Advantages:

- Instant Immobilization
- Smoother membrane profles
- Superior presentation of cell components
- Enhanced immunoreactivity
- Eliminates artifacts due to chem. fixation

Disadvantages:

- Only appropriate for small / thin samples
- Low contrast of membranes
- Freezing artifacts
- Longer sample preparation time

Sample Freezing methods

Jet Spray

Very small samples such as viruses

Cold block (Slamming)

Tissue pieces

Plunge

Very small samples like viruses
Tissue pieces
Single cells in agarose

Primary Disadvantage:

2-20μm freezing depth

High pressure

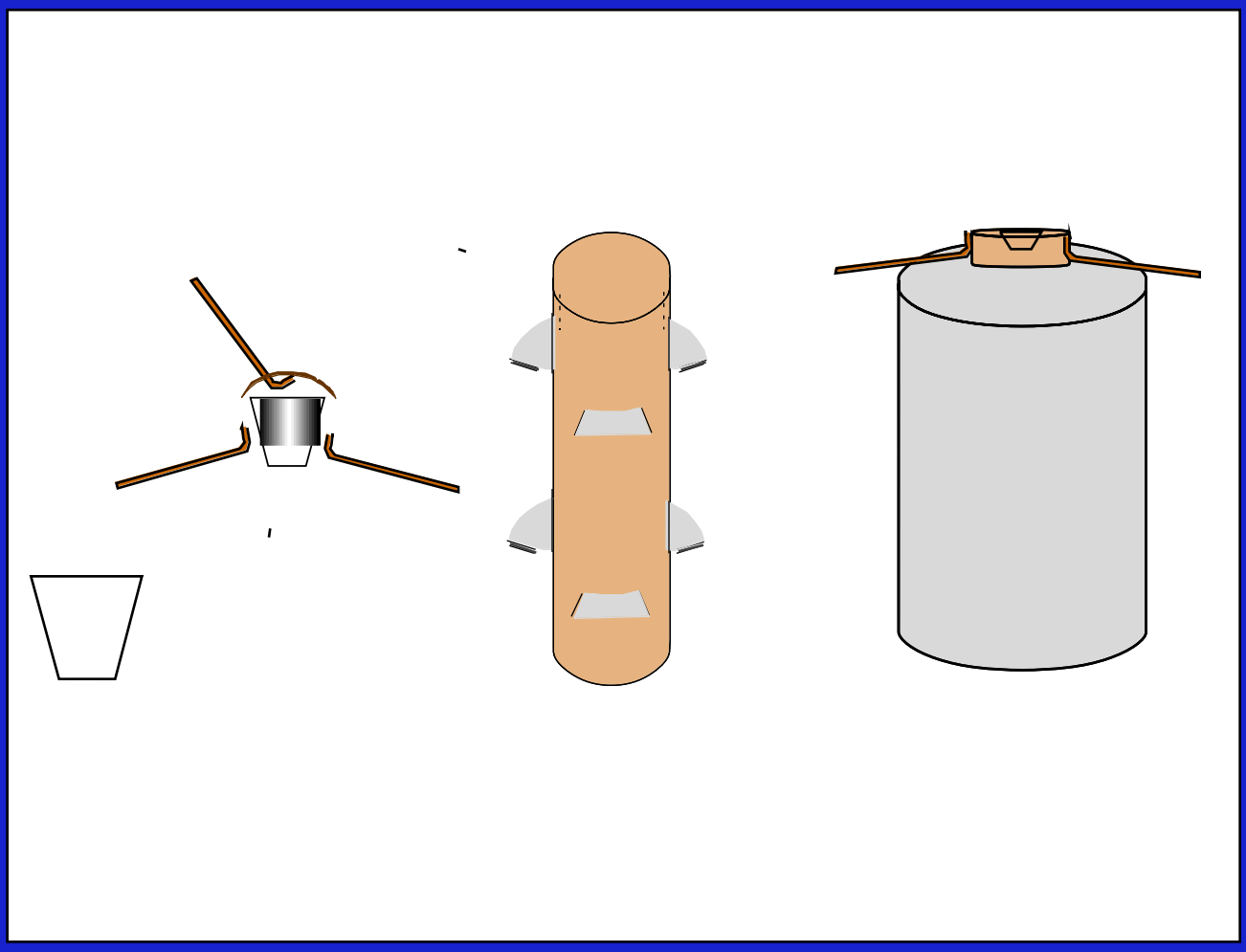
Primary Advantage:

200μm freezing depth

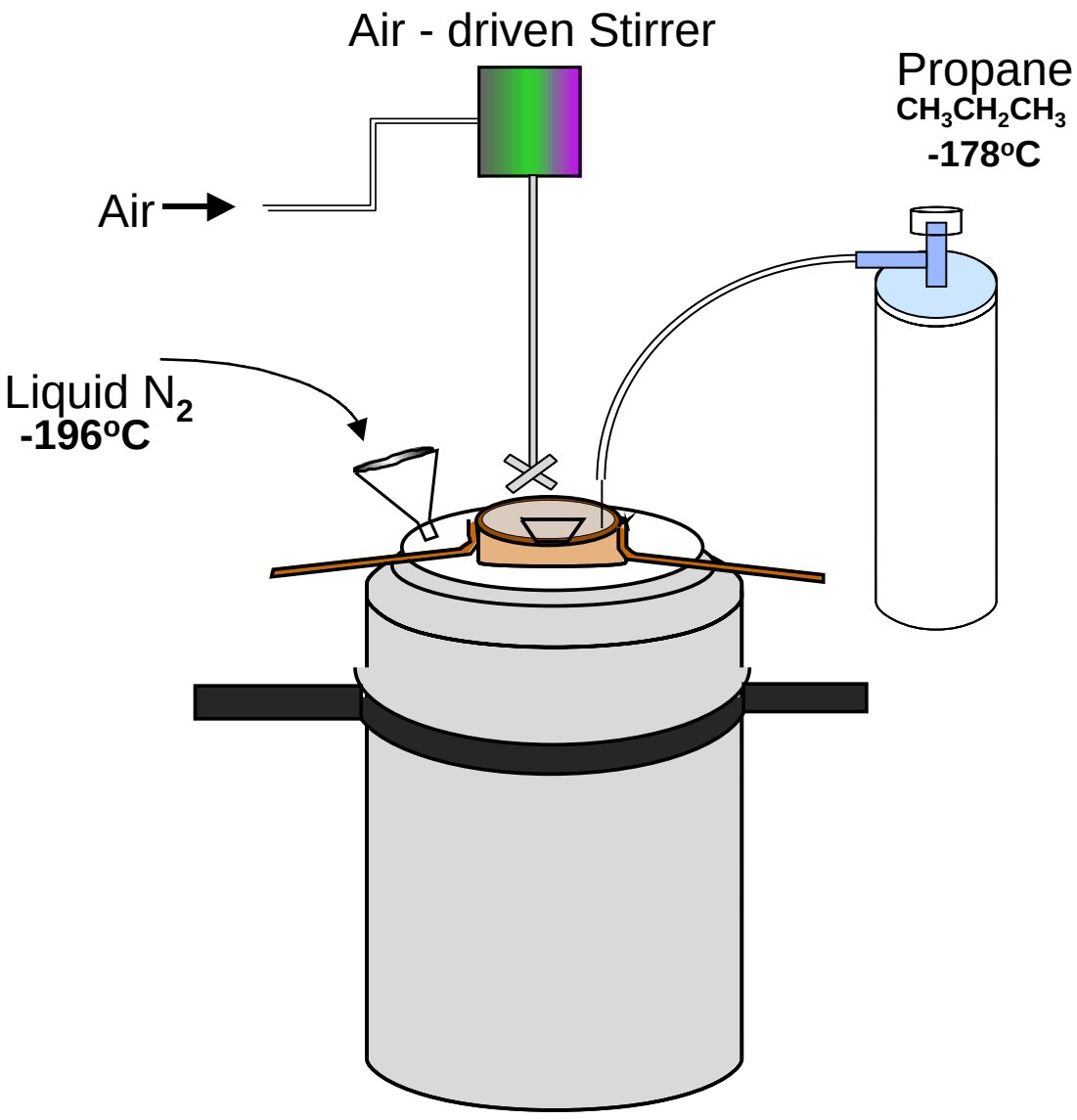
Primary Disadvantage:

High cost of instrumentation

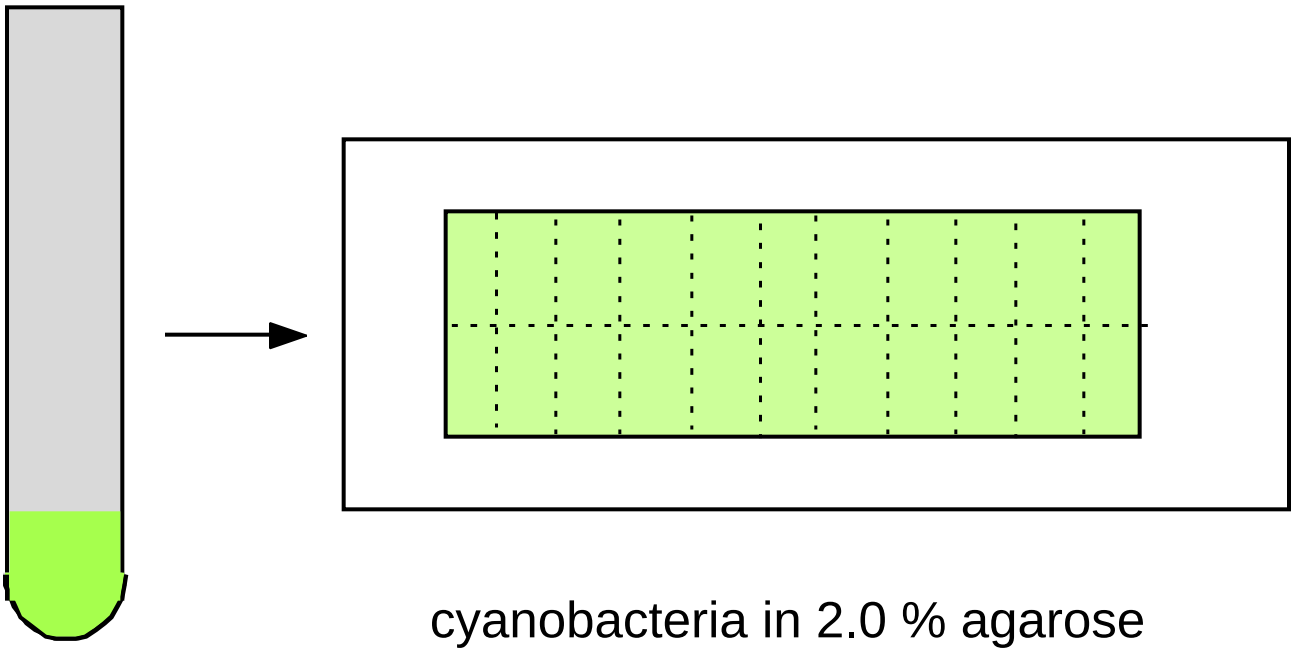
Plunge Freezing
(at Purdue)



Plunge Freezing Apparatus



Embed cells in agarose sheet



cyanobacteria in 2.0 % agarose

Vitrification of Biological Samples

Rate of freezing critical to prevent ice crystal formation

At 1 atm cooling rates of >100,000^{o}C/s are necessary

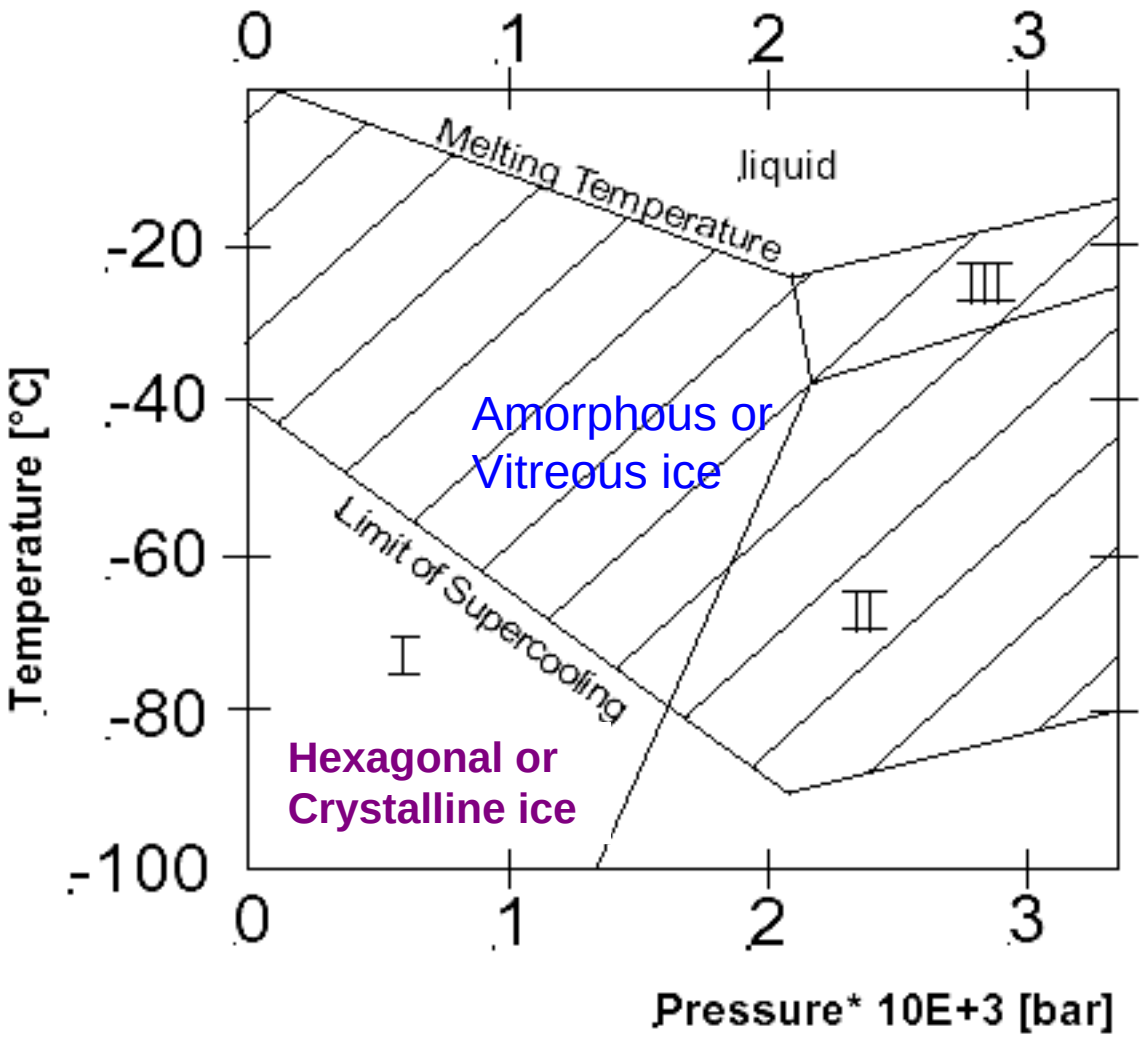
Plunge freezing: **Thin film-okay**
Tissue-2-5μm

Slam freezing: **10 to 15μm**

Jet freezing: **40μm**

At 2000 atm cooling rates of ~200^{o}C/s are necessary

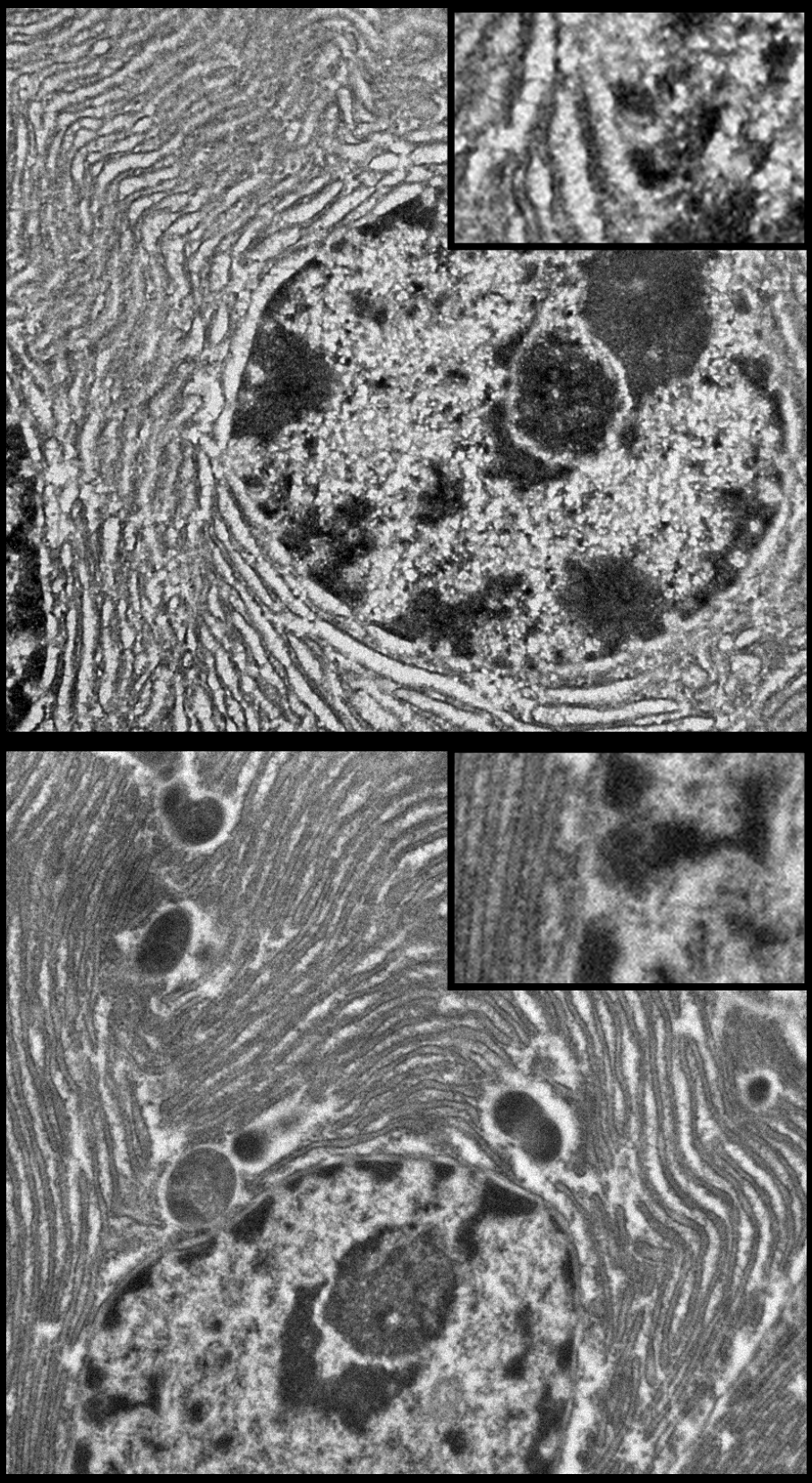
High pressure Freezing: 200+μm



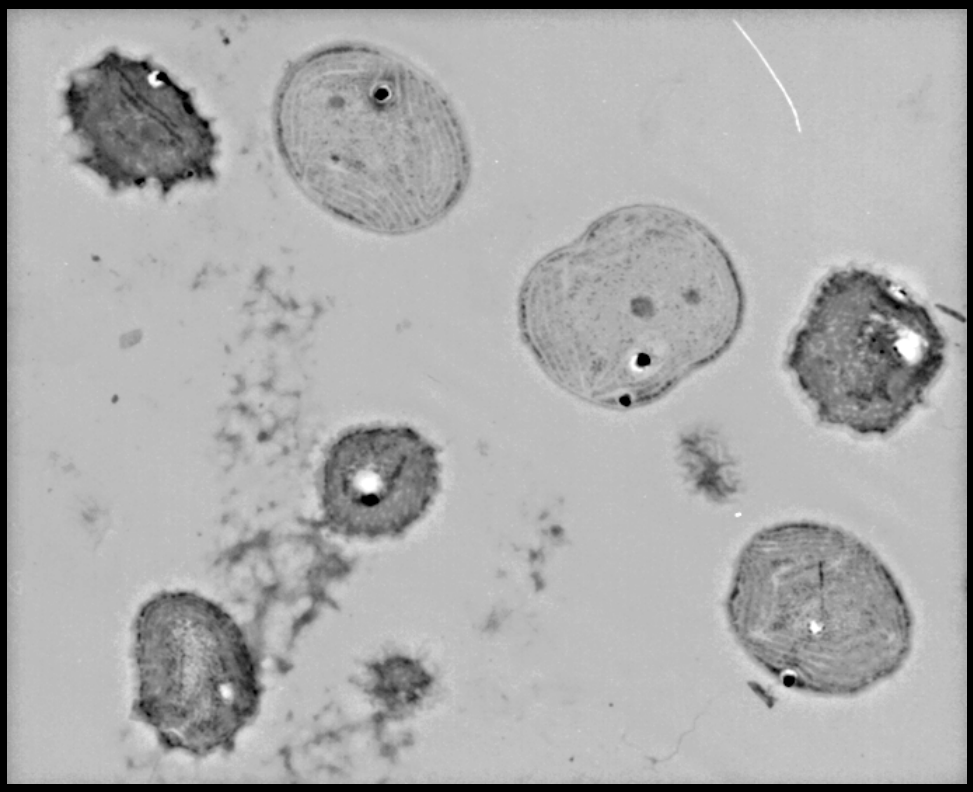
H_20 Phase Diagram

1 Supercooling capability curve
2 Melting point curve

Pancreas-plunge freeze +FS



Freezing Artifact
Cyanobacteria



Freeze - Substitution at -85°C

- **Ultrastructure**
  - Acetone
  - Acetone + OsO_4
- **Immunocytochemistry**
  - Ethanol (ETOH)
  - Methanol (MEOH)
  - Acetone

solution changed every 24 hrs for 4 + days

Warm-up from -85°C

- insulated box with layer of dry ice

- Gradual

  ~18 hours to -20°C
  2 h at +4°C
  additional 2-4 h at RT for
  OsO_4 treated

Resin Embedding

- **Ultrastructure**

  Spurr's Resin

  Epoxy resin mixture

  Low viscosity

  Polymerize at 60^{o}C

- **Immunocytochemistry**

  LR white or LR Gold

  hydrophilic, polar

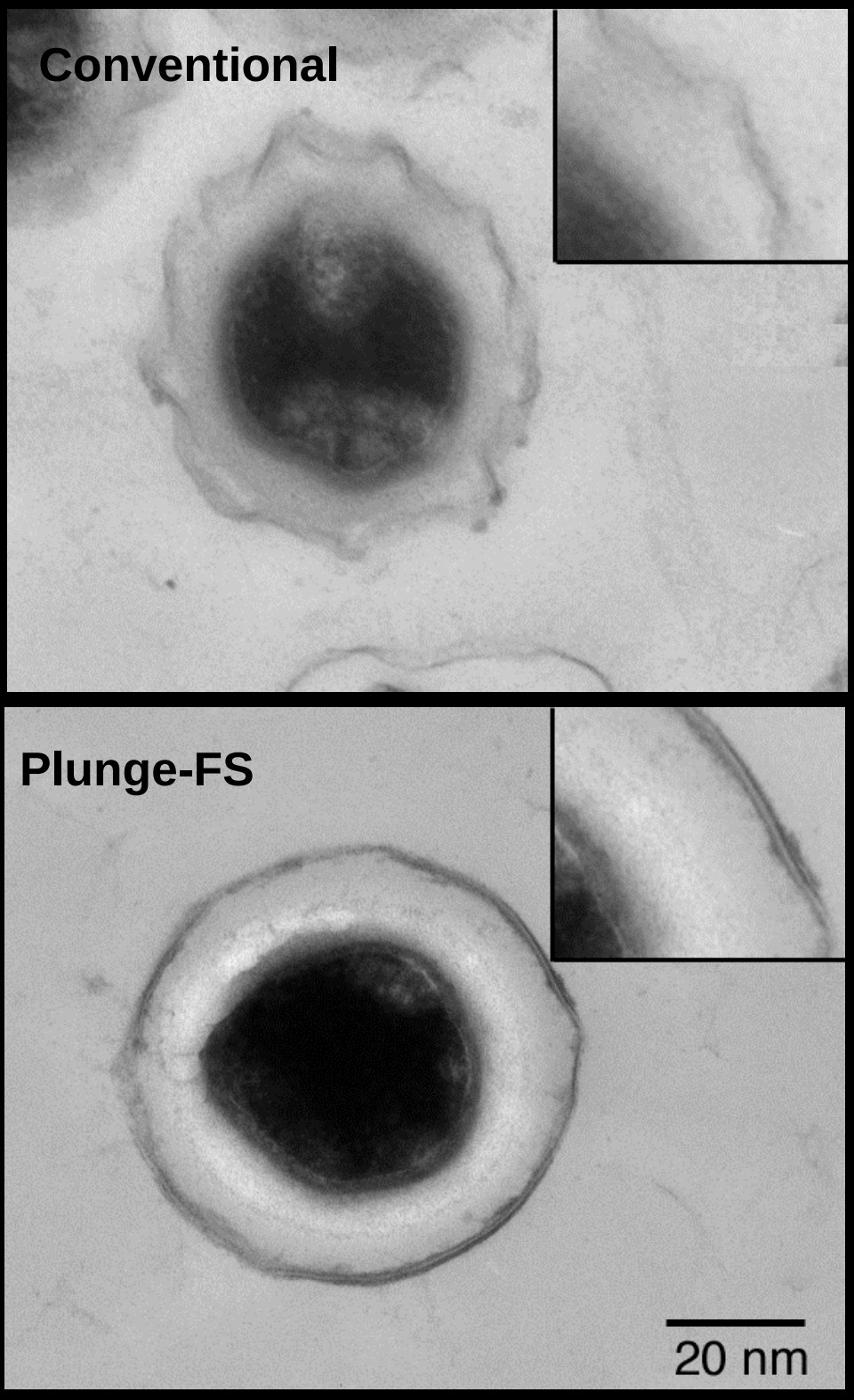
  Lowacryl

  K4M-hydrophilic,polar

  HM20 -hydrophobic, nonpolar

Leica Freeze-substitution Unit

Bacteria Spore



FREEZE-SUBSTITUTION AND IMMUNOCYTOCHEMICAL PREPARATION OF CYANOBACTERIA

Cells spun down, resuspended in 2% agarose and spread in thin sheet on glass slide

4mm squares **FROZEN** by plunging into liquid N_2-cooled propane

FREEZE-SUBSTITUTED to replace H_2O with ETOH at -85˚C

INFILTRATED with acrylic resin Lowicryl HM20 & **POLYMERIZED** with UV light at 4˚C

Thin **SECTIONS CUT** and picked up on formvar-coated Cu or Ni grids

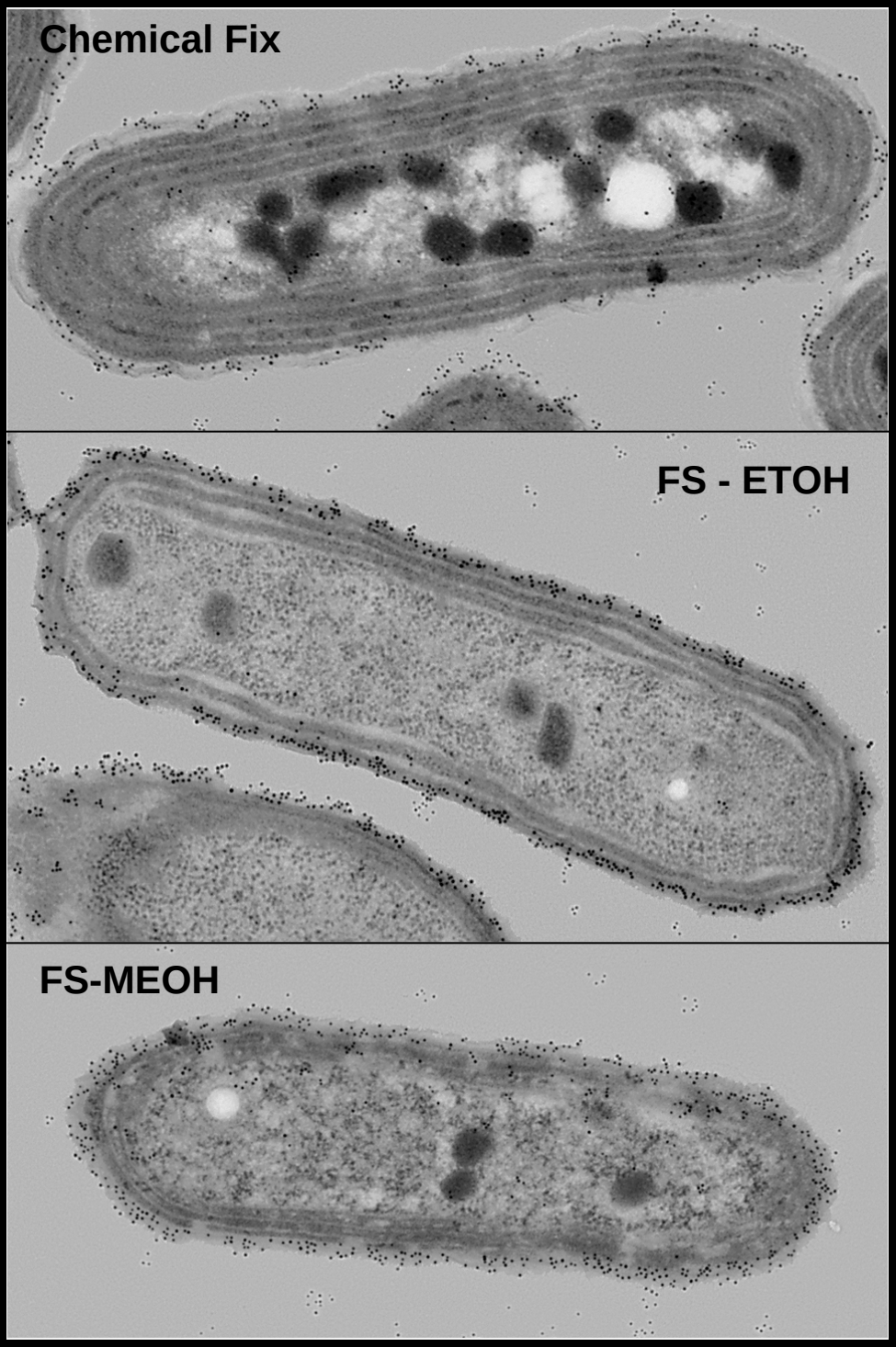
Grids incubated in **PRIMARY ANTISERA** for 15h at 4˚C

Incubated in **SECONDARY ANTISERA** (IgG) conjugated to 10nm colloidal gold

STAINED with uranyl acetate and lead citrate

VIEWED with a transmission electron microscope

Cytochrome Oxidase in Synechococcus sp.PCC7942



Cyanophycin label in Cyanothece