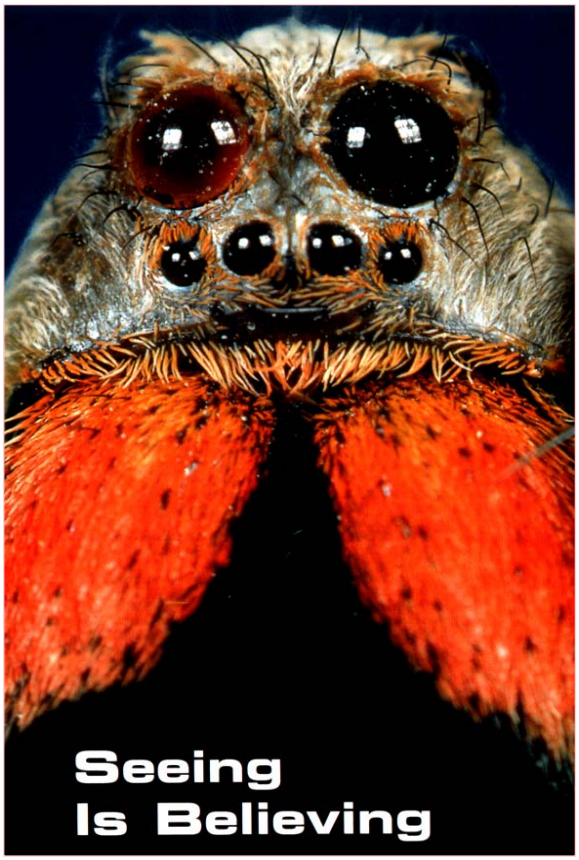
Transmission Electron Microscopy (TEM) in Biology (Biosciences)

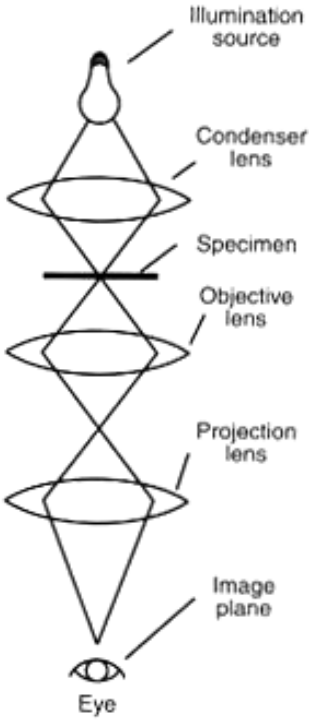
Chair of Cell & Tissue Biology
Center for Electron Microscopy
Aleksandra Korac

University of Belgrade, Faculty of Biology
School of EM



University of Belgrade, Faculty of Biology
School of EM

Seeing with light!



All living organism are made of :

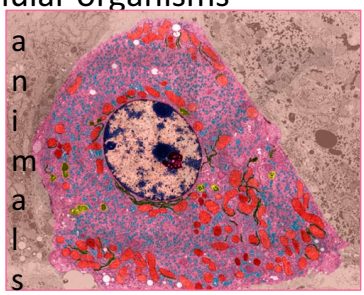
*proteins
*lipids
*carbohydrates
*nucleic acids
*water
*inorganic ions (Na, K, Ca, Fe, Cu…)

cells

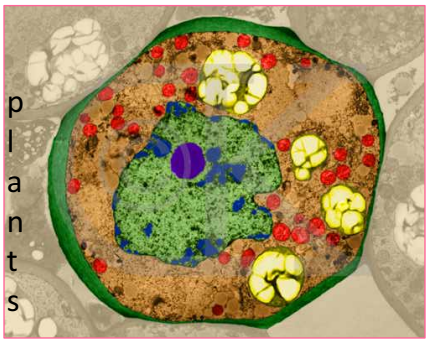
Single cell organisms

Multicellular organisms

animals



plants



*The main use of the transmission electron microscope is to examine in submicroscopic detail the structure, composition, and properties of specimens in ways that cannot be examined using other equipment or techniques.

University of Belgrade, Faculty of Biology
School of EM

Seeing with electrons!

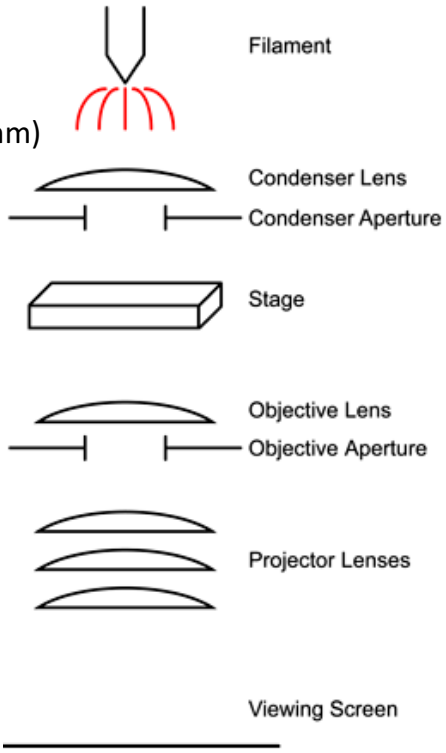
- Magnification is 10.000-100.000X
- Has a resolution 1.000X better than light microscope (0.5 nm)

*TEM works in much the same way as an optical microscope.

*Involves a high voltage electron beam emitted by a cathode and formed by magnetic lenses.

*The electron beam that has been partially transmitted through the very thin (and so semitransparent for electrons) specimen carries information about the structure of the specimen.

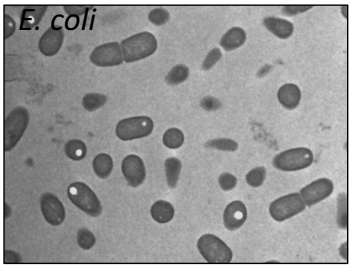
*The "image" is then magnified by a series of magnetic lenses until it is recorded by a fluorescent screen or CCD camera.

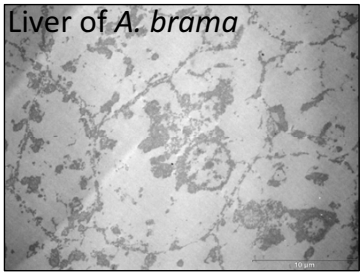


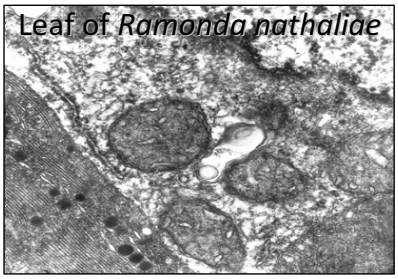
But, before we get a cell image we need to know:

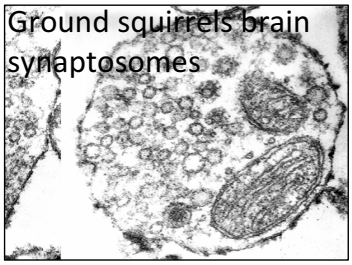
*Which specimen do we want or have? / extremely diverse samples

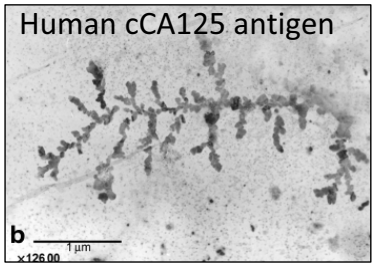
1. Cells
2. Tissue
3. Cell fractions
4. Isolated macromolecules/extracellular components









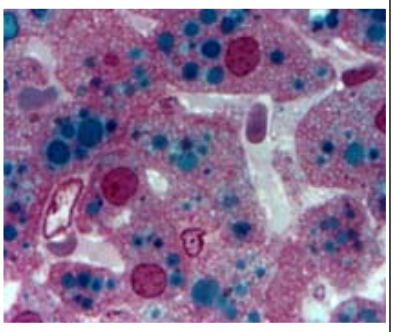


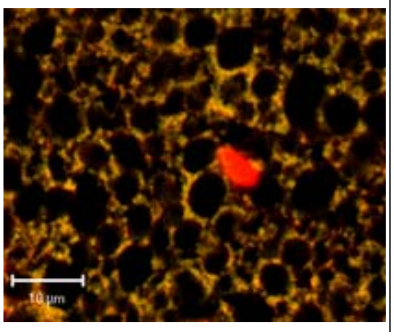
University of Belgrade, Faculty of Biology
School of EM

*What do we want to see?

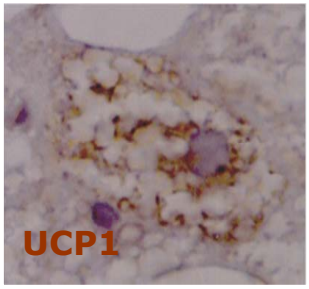
TEM image – ultrastructure –purely descriptive

Brown adipocyte



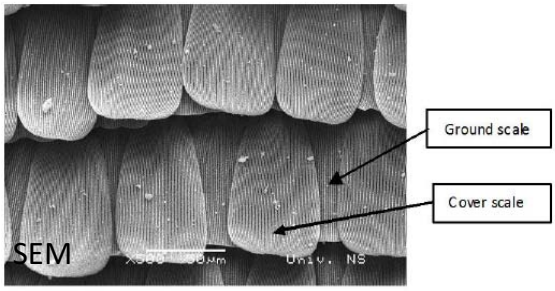


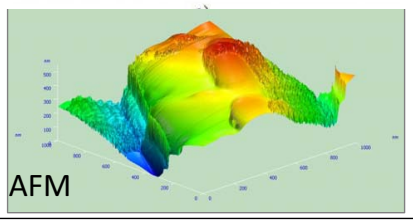
Missing!



TEM image - structural basis of specific biological function

UV reflection

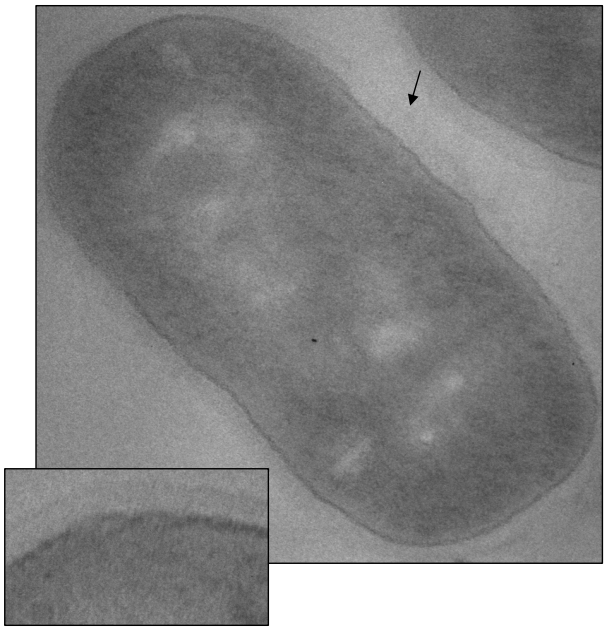




Missing!

University of Belgrade, Faculty of Biology
School of EM

TEM image – specific chemical composition



Routine TEM staining x88.000

Missing!

*Why specimen preparation is so important?

Most biological specimens are structurally weak, hydrated, and electron translucent. All of these features are the exact opposite of what is required of a TEM sample.

Initially it was felt that biological specimens could not be examined with EM due to the extreme conditions inside the TEM (high vacuum, intense heat generated by the beam of electrons, depth to which electrons can penetrate a specimen, etc.).

University of Belgrade, Faculty of Biology
School of EM

*Goals of Specimen Preparation

1) To observe the specimen in as near to the "natural" state as possible.

2) To preserve as many features of the specimen as possible. Interpretations drawn from incomplete preservation can lead to drastically incorrect interpretations of the data.

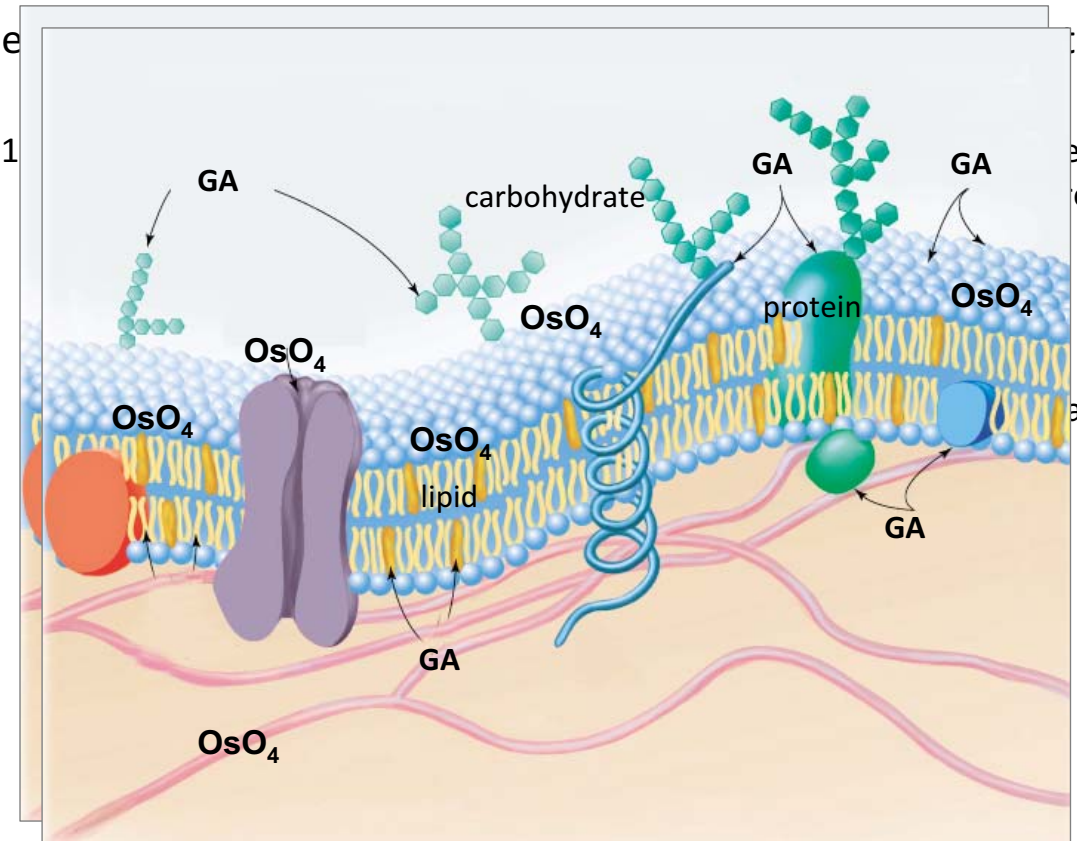
3) To avoid the introduction of artifacts that could obscure or influence our interpretation of the specimen. In addition to changes that might occur during the fixation some features that might have been initially preserved may be extracted during further processing. One tries to keep this to a minimum.

4) To render the specimen stable for examination in the TEM.

*The the TEM

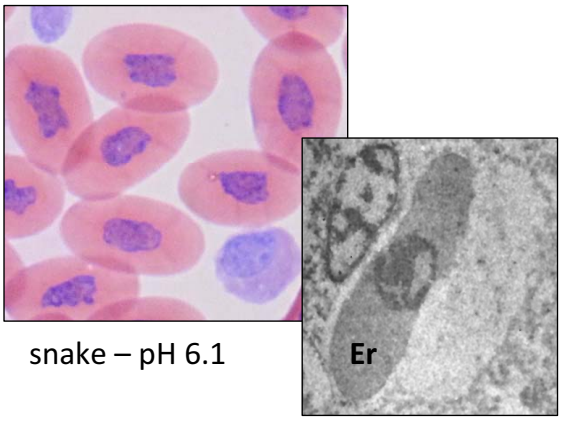
1 ellular
es to
must

ation:

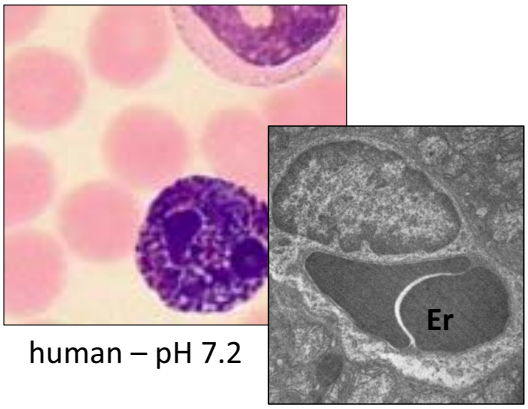


University of Belgrade, Faculty of Biology
School of EM

*A number of factors are influential in reducing the number of artifacts that are induced during the process of fixation. Some of these include changes in pH and/or temperature, osmotic potential causing osmotic shock, physical or mechanical damage.



snake – pH 6.1



human – pH 7.2

We have to adjust osmolarity of fixation solution to physiological value!

2) **Dehydr** ... es the exchan

3) **Infiltra** ... plastic suppor ... be cut off. Ini ... every portion ... terial. Depen ... a few to man

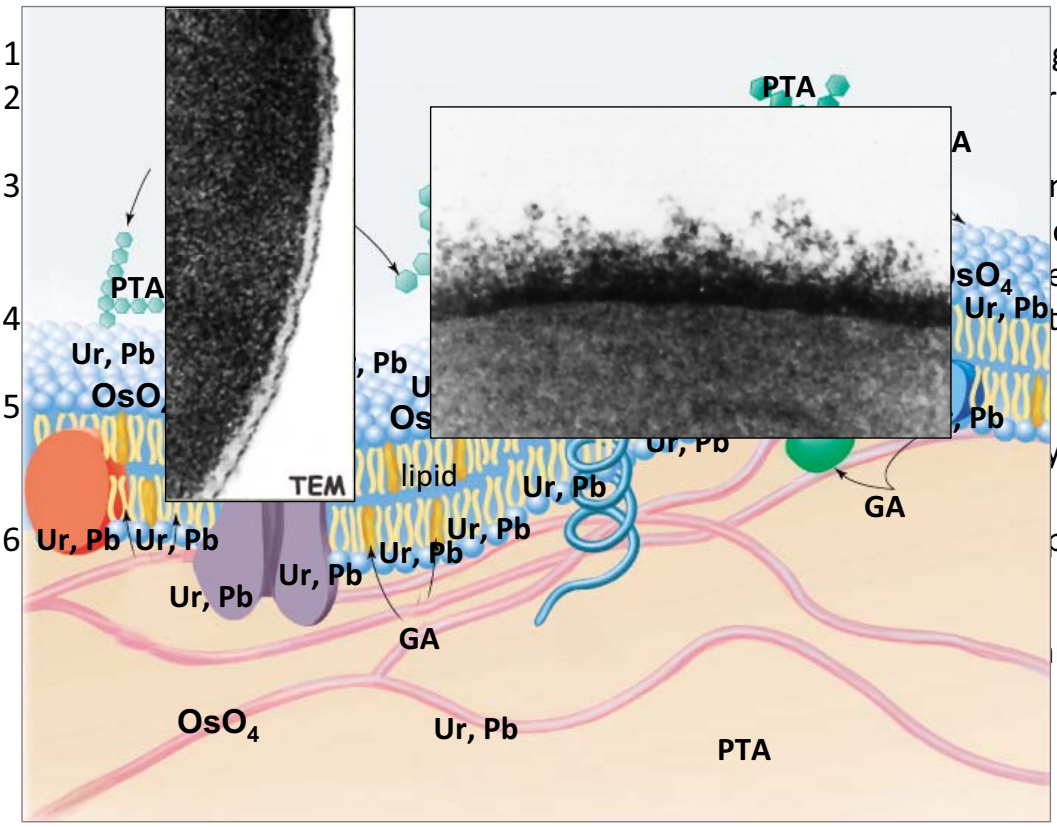
4) **Embed** ... esin it is plac ... en to accom ... let of cells, e

5) **Sectioning:** Ultra-thin (50-60nm) sections are cut from the polymerized block by using an ultramicrotome and a glass or diamond knife.

University of Belgrade, Faculty of Biology
School of EM

*Staining of Sections

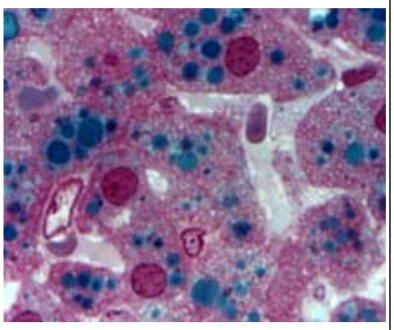
1 ... ges.
2 ... rasting
3 ... nts of ... ctrons ... eam.
4 ... tuated
5 ... metal ... y good
6 ... parted ... nd having

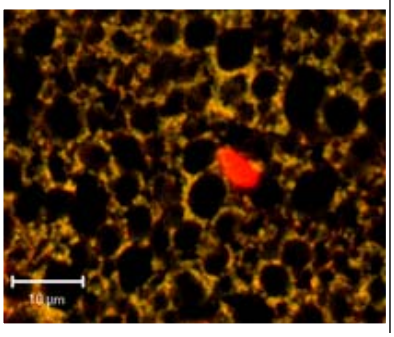


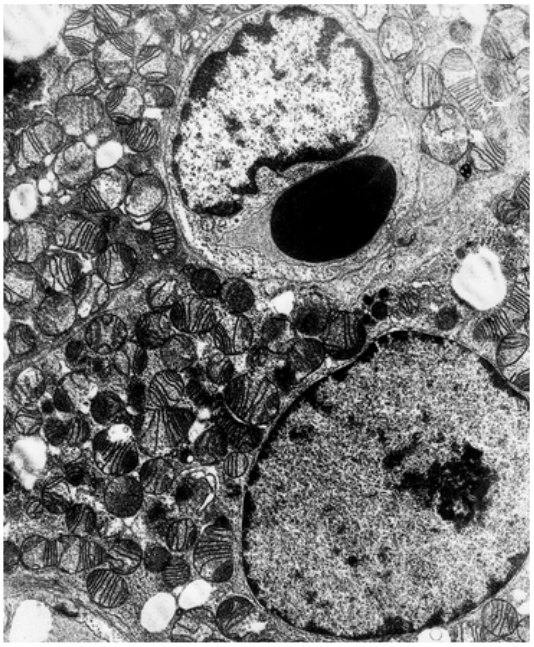
*What we get to see?

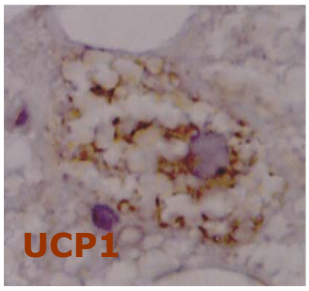
TEM image – ultrastructure –purely descriptive

Brown adipocyte





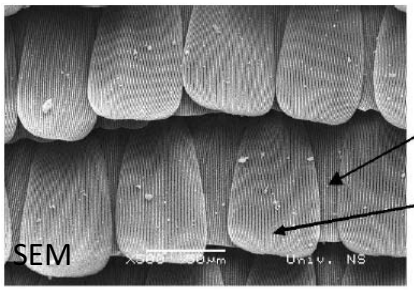


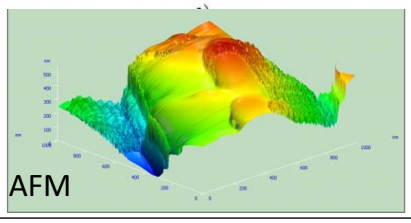


University of Belgrade, Faculty of Biology
School of EM

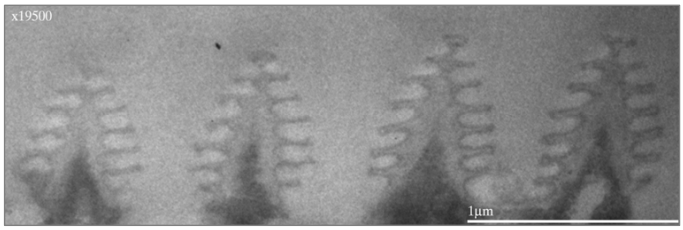
TEM image - structural basis of specific biological function

iridescence

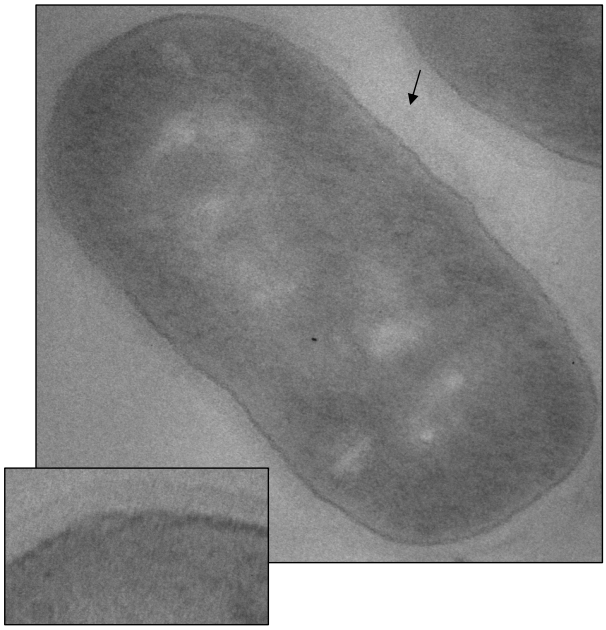




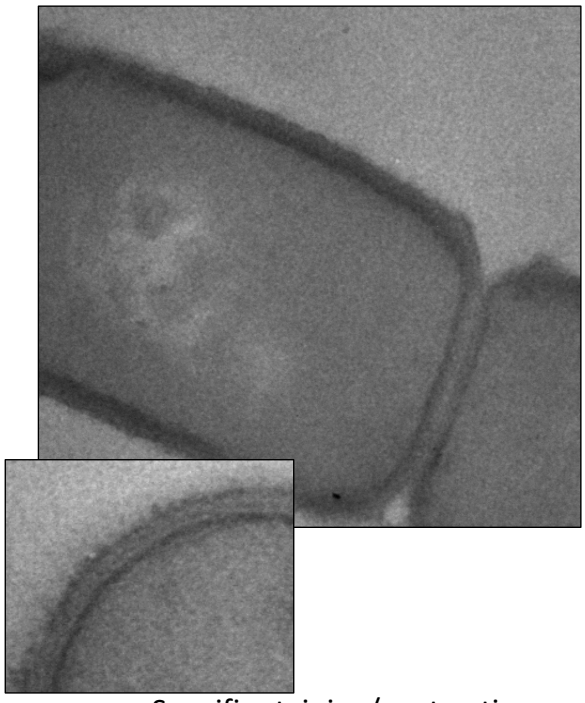
structural color



TEM image – reveals specific chemical composition

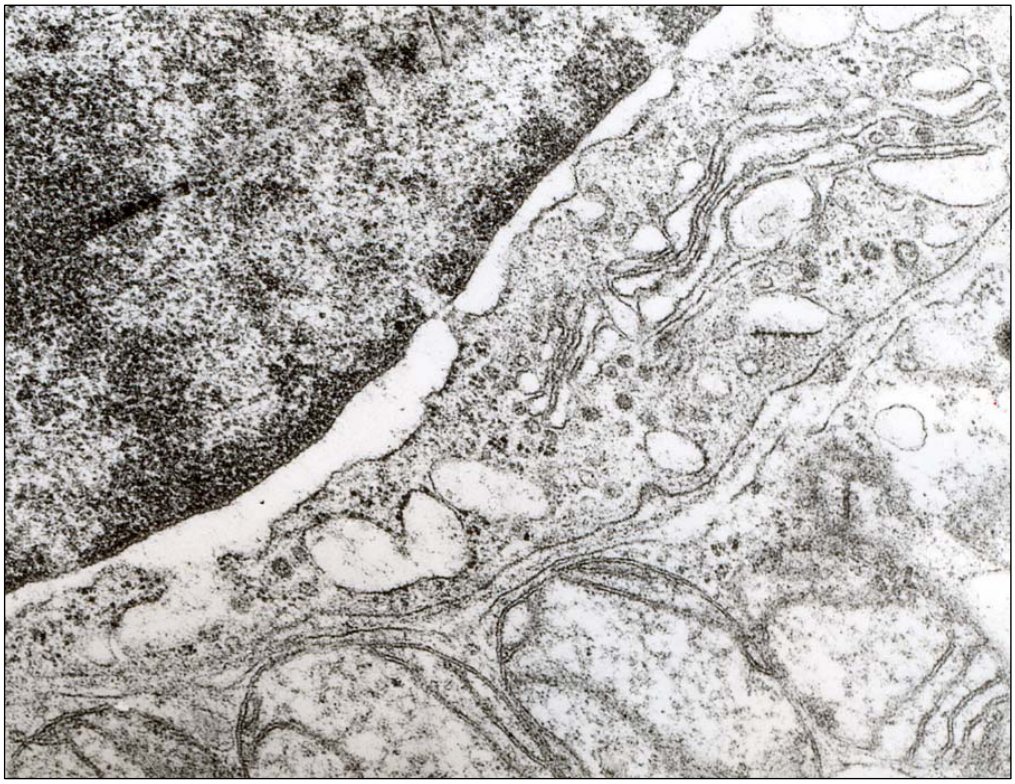


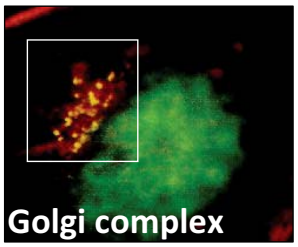
Routine TEM staining x88.000



Specific staining/contrasting

University of Belgrade, Faculty of Biology
School of EM





Chair of Cell & Tissue Biology
Center for Electron Microscopy

Aleksandra Korac, PhD
Vesna Koko, PhD
Maja Cakic- Milosevic, PhD

Mirela Ukropina, MsC
Ksenija Velickovic, PhD student
Milica Markelic, PhD student
Igor Golic, PhD student

Anita Lazarevic, BsC
Maja Bogdanovic, BsC

Jelena Radovanovic
Nada Serban

University of Belgrade, Faculty of Biology
School of EM

*It's all about learning/ guidance of our teachers!

Pierre Favard
Ivry-sur-Seine, Paris

Vesna Koko — Jelena Radovanovic — Nada Serban
Faculty of Biology, Belgrade

Pierre Gounon,
Université de Nice

Maja Cakic-Milosevic
Faculty of Biology, Belgrade

Aleksandra Korac
Faculty of Biology, Belgrade

Mirela, Ksenija, Milica, Igor
Faculty of Biology, Belgrade

Waltraud Klepal
University of Vienna,
Austria