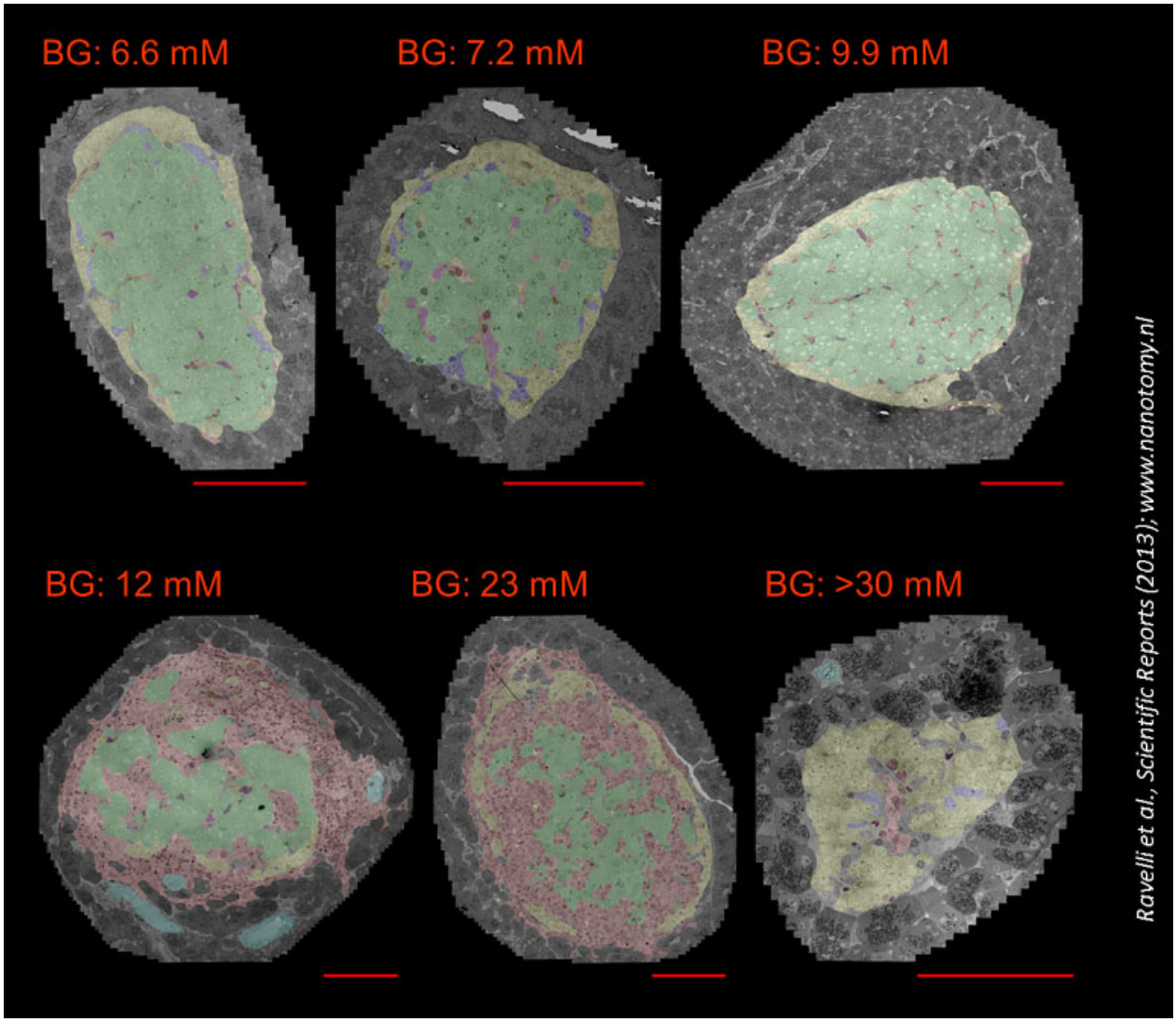
Nanotomy, T1D, electron microscopy



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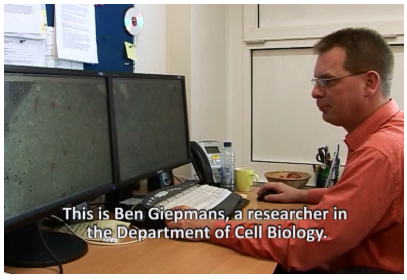

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Short version!

Assignment 1: Electron microscopy structure/function

A cell contains organelles that are essential for its function. Depending on cellular function, one type of cell will have a higher number of certain organelles than others. To check if you know the various cell organelles, examine the following schematic drawing of a cell (an exocrine cell).

a. Identify the various cell organelles by placing the right number at the right line
b. State the main function(s) of the organelle in the table

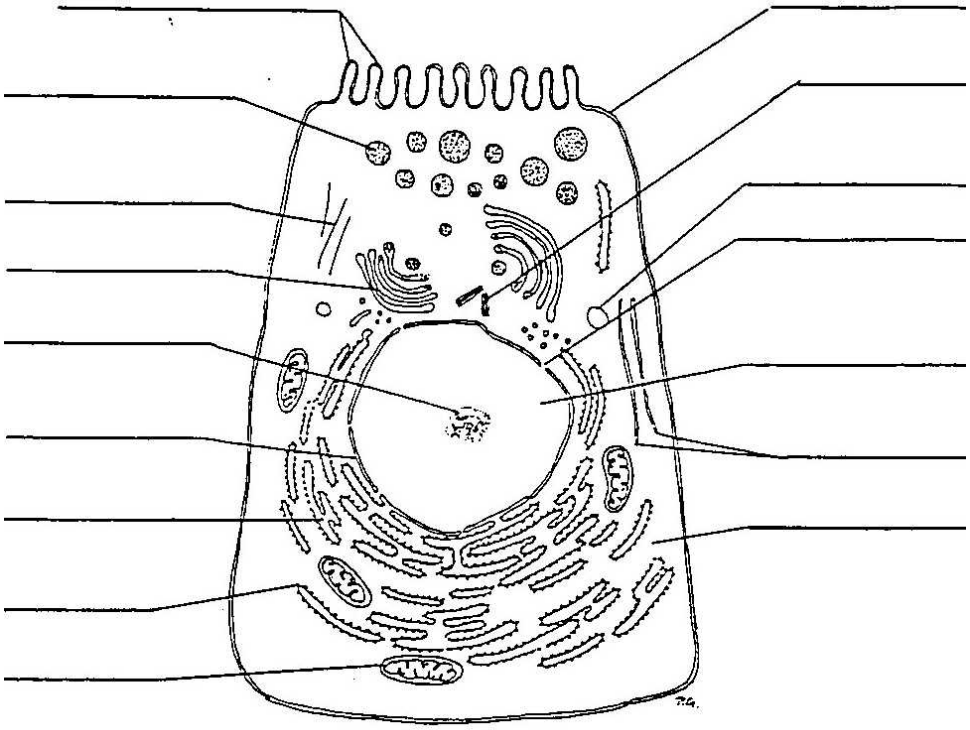


Fig. 1. Source: *Laboratory Manual of Histology,* Pappas. (W. C. Brown, 1990)

Structure	Function	Structure	Function
1. centriole		9. microtubules	
2. cytosol		10. mitochondria	
3. Golgi complex		11. microvilli	
4. nucleus		12. nucleolus	
5. nuclear envelope		13. plasma membrane	
6. nuclear pore		14. ribosomes	
7. lysosome		15. rough endoplasmic reticulum	
8. microfilaments		16. secretion drops	

Electron microscopy

In ***transmission EM (TEM)***, a high voltage generated between a heated cathode (incandescent filament) and an anode produces a beam of electrons. One or more condenser lenses focus this beam onto the plane of focus of the objective lenses, where an ultrathin specimen section which can be irradiated has been placed. The objective lenses create a magnified image of the object which is shown on screen or captured by camera. A standard TEM provides magnification of up to 300,000 times, with a resolution of ~ 2 nm, of specimen sections which are ~ 60 nm thick, with a maximum diameter of 3 mm.

The biological material present in the ultrathin section mainly comprises C, H, N and O and does not scatter electrons sufficiently to provide an image, which is why the specimen must be stained with heavy metals, which do scatter electrons. The most common contrast medium is osmium tetroxide (OsO_4), which binds particularly easily to double bonds of lipids, fixing them by creating cross-links, making membranes visible.

In ***scanning EM (SEM)***, the electron beam is focused by the condenser and objective lenses in the same manner as in TEM. Here, too, the specimen is placed in the focal point. The primary electron beam is not stationary, like in TEM, but scans it in a grid-like fashion. The electron beam scans the specimen surface line for line, releasing secondary electrons (SE2) in the sample or having the electrons reflected (backscatter electrons). These are both used to create an image of the specimen surface. If the specimen section is ultrathin, the electrons will of course pass through it. By placing a detector underneath, a TEM image can be transformed into an SEM image. This is known as ***STEM: scanning transmission EM.***

Background: Essential Cell Biology, Alberts et al., (Garland, 4th ed. 2013) page 11.
*Assignments 1-2 have been modified from Drs. D. Opstelten & N.A. Bos, Manual for Cell Biology

Assignment 2: Nanotomy, EM of tissues, cells, organelles and macromolecules

Nanotomy is an innovation in EM that allows to study tissues, cells, organelles and macromolecules in Google-Earth-like fashion. Here, nanotomy has been used in an animal model for Type 1 diabetes: Ravelli et al. (2013); www.nature.com/srep/2013/130508/srep01804/full/srep01804.html.

Type 1 diabetes (T1D) is an auto-immune disease resulting from degradation of the insulin-producing beta cells, which are located in the islets of Langerhans in the pancreas. The trigger is unknown, a cure does not exist, and patients depend on lifelong insulin therapy.

Study ultrastructure. Go to http://www.nanotomy.org/islets2/isletVgrey.html . This dataset can be studied in the same way you view a landscape in Google Earth. Click on the IIP icon at top left for extra instructions if necessary. Go through the annotations and answer the questions below. The numbers correspond to the annotations. If you place your cursor on 'Supracellular', for example, a submenu will appear, including A, Islet. ***Before you begin, drag the menu at bottom left up a bit and the scale will appear.***

1. Islets of Langerhans in recent-onset type 1 diabetes (rat)

Judge what the largest structure is that you can recognize. And if you zoom in, what is the smallest? What are the dimensions?
Once again: ***The annotation menu can be dragged to allow the scale to show.***
Largest: Size is approximately:
Smallest: Size is approximately:

1A. Name the clearest differences which distinguish the islets of Langerhans from the exocrine pancreas.

What are the various functions?

1B. Which cell is in the capillary?

1C. Which two types of cell do you recognize in the vein? What are the most obvious differences?

1D. The centroacinar lumen belongs to the:

a. endocrine pancreas and contains enzymes
b. endocrine pancreas and contains hormones
c. exocrine pancreas and contains enzymes
d. exocrine pancreas and contains hormones

1E. Depicted here is a cross-section of a bundle of unmyelinated axons. The bundle was discovered more or less by chance: the electrons cause the axons to be slightly lighter. These contain round tubules and light-grey filaments. How many axons do you see here?

1F. In some exocrine cells the nucleus is not visible. Why?

2G. Inflammatory cells are present because there is an ongoing immune response to the islets in the rat. What type of leukocyte is visible here? How can you see this?

2H. What is the approximate size of this erythrocyte?

2I. What is typical of the nucleus of a monocyte?

2J. This phagocyte is (a) passive ***or*** (b) active, because:

2K. The granulocytes in the blood cell practical were spherical / round. These clearly are not. Explain the difference?

2L. The leukocyte has black spots on it. What is their approximate size? What could they be?

2M. The small platelets clearly have a more heterogeneous content than the adjacent erythrocytes. How many platelets are visible in this vein?

3A. The rough ER is important among other things for:

The black spots measure approximately ..... nm. These are ..... on the inside / outside of the rough ER.

3B. A mitochondrion is easily recognized by:

3C. The cell nucleus contains:

Several types can be distinguished and these are:

With regard to function, this reflects the process that we call:

3D. The Golgi apparatus can be nano-anatomically distinguished from the ER because it:

The Golgi apparatus is important for such things as:

5. Structure / function of vesicles

5A. Dense bodies are known as such because:

5B. Lysosomes play an important role in:

5C. What type of cell is this which is swarming with caveolae?

5D. These are about the smallest vesicles in existence. What is their diameter?

5E. And what is the diameter of the lipid droplets?

5F. Which two characteristics allow this to be recognized as an early endosome?

5G. Clathrin-coated pits are characteristically involved in (a) endocytosis ***or*** (b) exocytosis.

5H. If the multi-vesicular bodies fuse with the plasma membrane, it is conceivable that:

6A. Crystae are typical of:

6B. Which atom is accumulated in this membranous mass?

6C. Do you recognize the cells? The fenestrae enable:

6D. The basement membrane depicted here is between two types of cells, which are:

6E. Here, the basement membrane forms part of a complex structure. This is still the diabetic rat. Morphologically speaking, both cells containing nuclei clearly appear to be leukocytes. However, these are in different locations. The leukocyte on the left is in the .... , while the other clearly is not. Explain what may be going on.

7. Macromolecules are just barely discernible at these image settings. There are certain characteristics which allow the various macromolecules to be recognized.

7A. How many nuclear pores can you distinguish in the ENTIRE cross-section of the nuclear membrane?

7B. This is the tip of the nucleus where nuclear pores can also be distinguished. How many are there?

7C. Polysomes comprise:

7D. Sketch a model of a single polysome with 5 ribosomes. If possible, indicate the 5'UTR and 3 'UTR and sketch a diagram of proproteins.

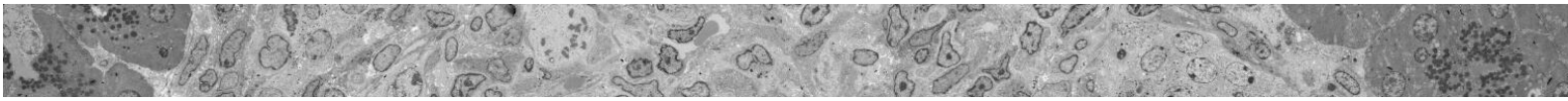
7E. Desmosomes are specialized cell-cell contact points, which in particular are important for (a) tissue strength ***or*** (b) forming a barrier.

7F. Tight junctions are specialized cell-cell contact points, which in particular are important for forming a barrier. Other than in desmosomes, there is no major concentration of intermediate filaments on the cytoplasmic side. Which barrier has been created here?

7G./H. Collagen is (a) cytoplasmic ***or*** (b) extracellular and serves particularly to:

7I./7J. Centrioles are often found perinuclearly and are mainly made up of:

7K. Every cell has a pair of centrioles. Give a rough estimate of how many centrioles should be visible in this dataset. Explain.



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