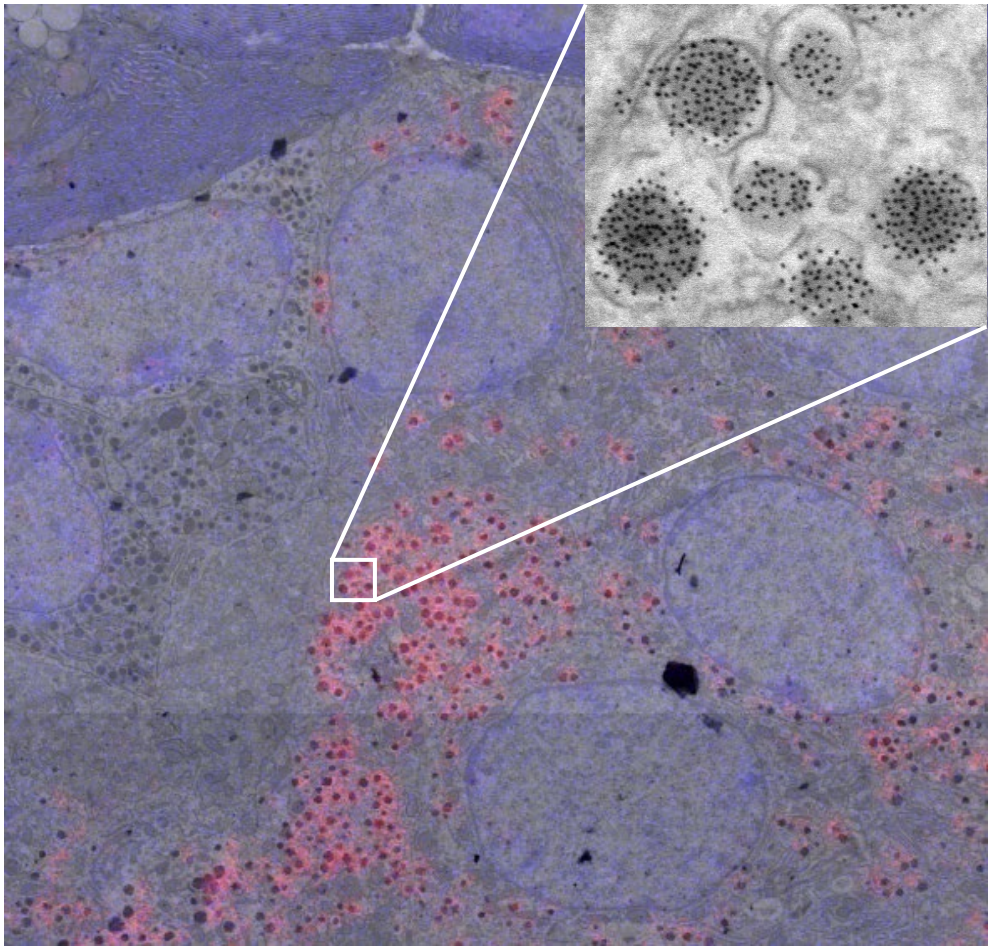
Cellular Analysis with EM



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Workshop and data based on original material (with permission) by:
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Introduction

This workshop is part of the course High-Resolution Imaging (NB4020) at TU Delft and complements the lectures and demo's on Scanning Electron Microscopy. In the lectures, students should get a basic understanding of how electron microscopes (EMs) in general, and scanning EM's in particular, work and how they can be applied in biology. Demonstrations serve to illustrate operation and applications of SEMs. The aim of this workshop is to make students familiar with EM data of biological tissue and to learn recognize various biological structures within the tissue. It also illustrates several novel TEM- and SEM-based applications, such as large-scale EM and correlative imaging.

The workshop consists of several parts with self-study (homework) assignments. It uses online data, so computer with internet access is needed. Some sources for further reading are contained at the end of this document.

Goals

After this workshop, students should be able to:

1. recognize and interpret electron microscopic images, with regard to basic tissue characteristics, cell types, organelles, and macromolecular complexes.
2. understand and explain how contrast is generated in these images.
3. understand the possibilities and limitation of large-scale SEM

Grading

Students work in couples. The assignments in this workshop should be worked-out and handed in via Brightspace (Assignment 2). Grading will result in Pass (sufficient score) / Fail (insufficient score) basis. The assignment will be further discussed in class. One question in the exam will relate to knowledge acquired during this workshop.

Additional reading:

Ravelli et al.; Scientific Reports (2013): www.nature.com/srep/2013/130508/srep01804/full/srep01804.html
(open acces)

De Boer, Hoogenboom, Giepmans; Nature Methods 12 (6) 503-513 (2015)
(available on Brightspace)

Part 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).

Assignment:

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

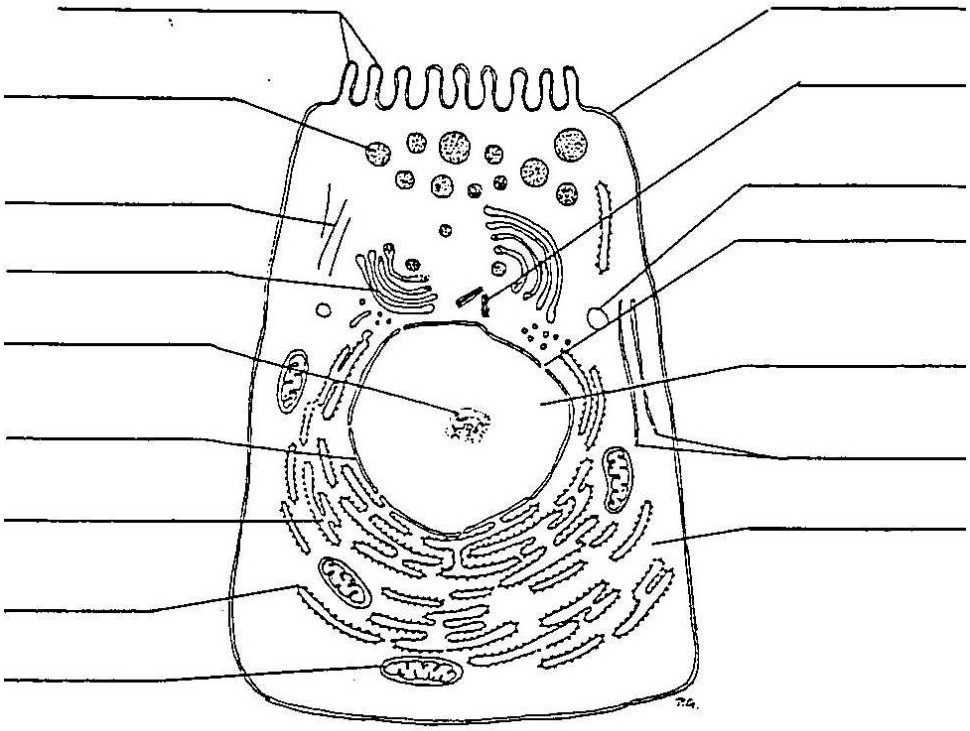


Fig. 1. Source: *Laboratory Manual of Histology,* Pappas. (W. C. Brown, 1990)

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	

Part 2: From tissue to molecular complexes

In this workshop, we will look at tissue material from a rat suffering from type 1 diabetes (see Ravelli *et al* in the further reading section). Type 1 diabetes (T1D) is an auto-immune disease that results in degradation of the insulin-producing beta cells (Fig. 2), which are located in the islets of Langerhans in the pancreas (Fig. 3). A cure does not exist; patients depend on lifelong insulin therapy. Moreover, the trigger that causes the disease is also unknown. Finding alternatives for insulin therapy and making advances in etiology of T1D benefits from a full structural and functional insight into Islets of Langerhans. With microscopy techniques samples can be studied at different magnifications. Typically, light microscopy covers large fields of view, see Fig. 3, a histological depiction of the islets of Langerhans. With EM, Islet morphology can be studied at the highest possible resolution. However, EM only provides gray-scale contrast which means that features are to a large extent recognized based on their structural appearance. Moreover, EM traditionally only provided selected snapshots. This makes data susceptible to user interpretation and bias, and data then also lacks the full structural context. In the assignments below, we will look at a section of a single islet.

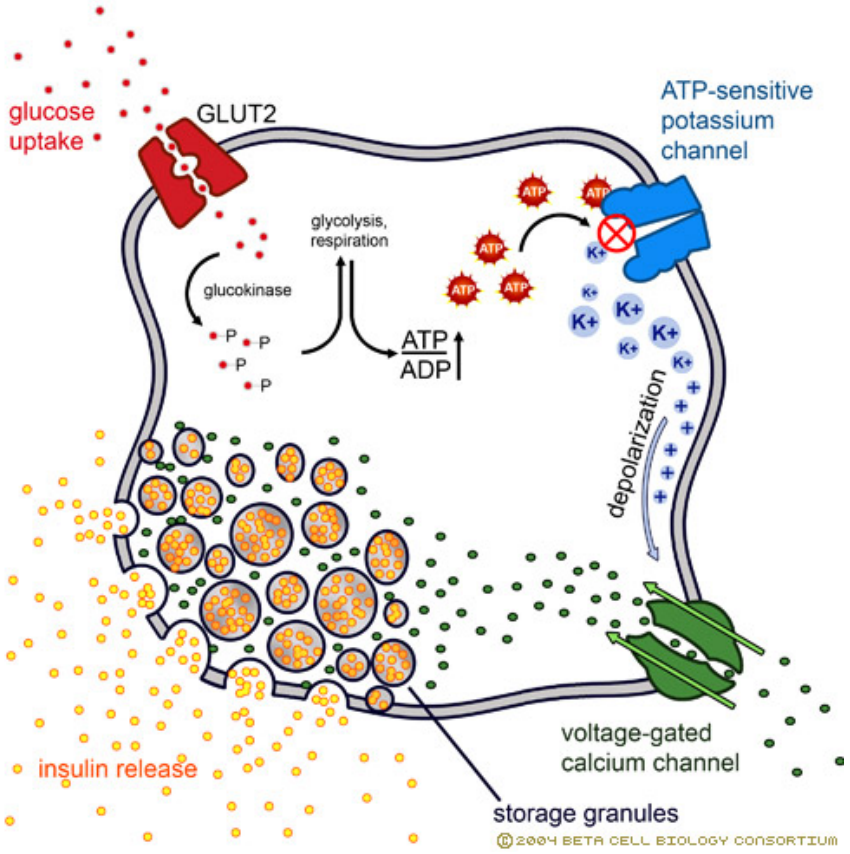


Fig. 2. Insulin secretion in beta cells caused by the increasing blood sugar levels.
Uptake of glucose by GLUT2 and glycolytic phosphorylation of glucose causes the ATP:ADP ratio to rise. This inactivates the potassium channel which depolarizes the membrane so that a voltage-dependent calcium channel opens. The increase in the calcium concentration leads to the release of insulin
Source: www.betacell.org

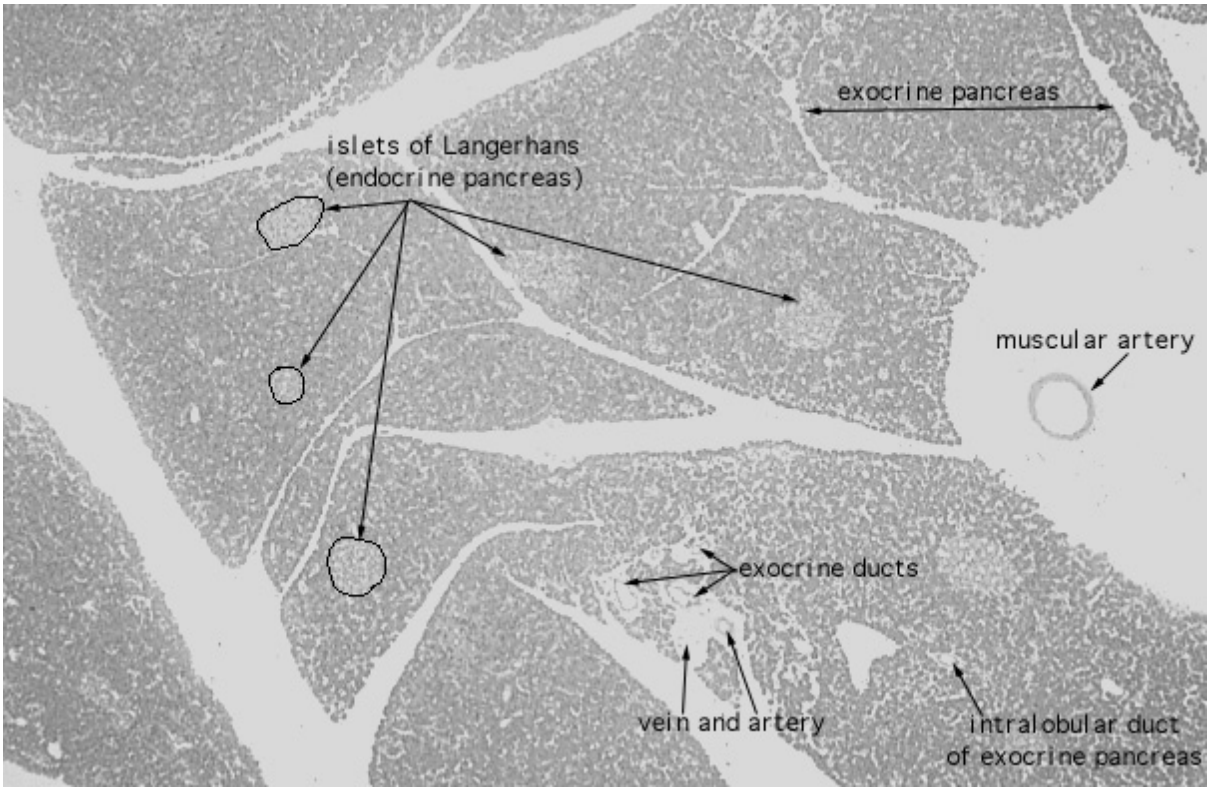


Fig. 3. The beta cells grouped in the islets of Langerhans in the pancreas. The rest of the pancreas consists of exocrine tissue where digestive enzymes are produced. The endocrine tissue – the islets of Langerhans – produces other hormones besides insulin.
Source: www.bu.edu/histology/p/10401loa.htm

Assignment 2.1:
Draw a scale bar in Figure 3, indicating the estimated dimensions. Try to indicate the scales of the voltage gated channel and the insulin storage granules in Fig. 2. Compare this with what you would fill in after completing all assignments.

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:

Study the ultrastructure of an islet of Langerhans. Go to www.nanotomy.org and select the dataset "*Islets in Type 1 diabetes*" ("*Ravelli (2013) data*" links to the same dataset). You can find further information on this dataset in the publication Ravelli et al. (2013): www.nature.com/srep/2013/130508/srep01804/full/srep01804.html. Click on the largest (grey) islet. 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. Different cell types, organelles, and even macromolecules have been annotated in the menu on the left. If you place your cursor on 'Supracellular', for example, a submenu will appear, including A, Islet. To aid in recognition, the corresponding structure is indicated in color in the thumbnail below the menu. ***Before you begin, drag the***

menu at bottom left up a bit and the scale will appear. Note that the scale adjusts when you zoom in and out.

Assignment 2.2
Go through the annotations and see whether you can recognize the different components in the EM data. Answer the questions 1-7 below. The bold numbers correspond to the annotations, for example **1A** refers to 1 Supracellular > A Islet.

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

(a) Judge what the largest structure is that you can recognize. And if you zoom in, what is the smallest? What are the dimensions?
Note: The annotation menu can be dragged to allow the scale to show.

Largest: Size is approximately:

Smallest: Size is approximately:

(b) Below the image, some acquisition data for this dataset is given. Can you estimate the total acquisition time needed? Can you explain the crenelated edges of the entire dataset? How has this sample been collected?

(c) What type of electron detector has been used for imaging? And what energy has been used? Could such data also have been collected with other electron detectors? If so, which, and argue whether that would need other electron energies or not?

(d) **1A**. Name the clearest differences which distinguish the islets of Langerhans from the exocrine pancreas.

(e) **1F**. In some exocrine cells the nucleus is not visible. Why?

2. Distinguishing cell types in the Islet.

(a) **2B**. The alpha cell produces glucagon, which is visible in the dark vesicles. Glucagon ensures that the blood sugar levels:

a. increase
b. decrease

(b) **2C.** The depicted beta cell is in bad shape: the rat has diabetes. Later you will compare the differences with a healthy rat. Do you recognize the various organelles? Only a few granules with hormones are visible, in particular to the bottom left of the nucleus. The crystal-like shape is typical and is even more pronounced in human beta cells. Which hormone is it?

(c) **2D**. Somatostatin-producing delta cells also form part of the islets, although they comprise only a few percent. We can distinguish various cell types thanks to the different granule structures. How can we differentiate between somatostatin granules and glucagon or insulin?

3. Organelles can be recognized on their structural appearance (see also Part 1)

(a) **3A.** The rough ER is important among other things for:

(b) The black spots measure approximately ..... nm. What are these? Are they located on the inside or outside of the ER?

(c) **3B**. A mitochondrion is easily recognized by:

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

4. During fixation cellular compartments can be fixed in different stages.

(a) **4C./4D./4E.** Exosomes are secreted granules. Using these various stages, is it possible to form a picture of exosome release? Yes/No

(b) In what way does exosome release differ from vesicle fusion in, for instance, insulin secretion?

5. Structure / function of vesicles

(a) **5A**. Why would Dense Bodies be called as such?

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

(c) **5E**. And what is the diameter of the lipid droplets?

6. The origin of contrast in the images

(a) **6B.** Notice the dark black contrast. How is the contrast formed / Which atom is accumulated in this membranous mass?

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

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

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

(c) **7K.** Every cell has a pair of centrioles. Give a rough estimate of how many centrioles should be visible in this dataset. Explain your answer.

Part 3: Islets during type 1 diabetes

Following this introduction to the EM of cells, organelles and macromolecules, we will now also briefly look at the effect of type 1 diabetes in the rat model and how this is apparent at the EM level.

Assignment: Return to the homepage (nanotomy.nl) and compare Dataset 1 (control) with Dataset 5 (diabetes). Answer the questions 1-4 below.

1. What is the blood sugar level of the healthy animal? And that of the animal with diabetes?

2. This is caused by a deficit in:

3. This is caused by the breakdown of beta cells. Insulitis clearly exists, since Dataset 5 shows many more:

4. The beta-cell destruction is clearly recognizable due to the following characteristics (name at least 3):

Two stages have now been shown. This should have given you an impression of how large-scale EM can help in understanding diseases. If you have time, you can further look at the other stages of which datasets are available on this site. This can also be done at home.

Part 4: Dealing with large-scale EM

In the previous assignments you have looked at large-scale EM data used to investigate diabetes. You have seen how cellular components and tissue characteristics can be recognized using biological knowledge.

Assignment: Read the methods section of the paper describing the dataset that you have investigated above: Ravelli et al. (2013): www.nature.com/srep/2013/130508/srep01804/full/srep01804.html. Answer the questions below

1. What was the total acquisition time for the dataset you worked on in Part2?

2. Estimate the total size (Gb) of this dataset.

3. Read the part in data annotation. Note that mind that all annotations and colourings that you have seen and used in this assignment have been made by hand, requiring visual inspection of the entire dataset! Describe in your own words what the authors mean by '*Atlases and text books have been a major source to define cell types based on morphology*'.

4. Large-scale and volume EM is rapidly growing in the past few years (see further reading, and there are also other examples available on nanotomy.org) and you can probably imagine that this manual analysis will become a bottleneck, in addition to bearing the risk of user bias. The authors also note this in the sentence '*Additional methods such as labelling should be applied in order to define all cell types unambiguously, as discussed in the main text*'. Briefly summarize in your own words the points in the discussion in the main text that the authors refer to here.

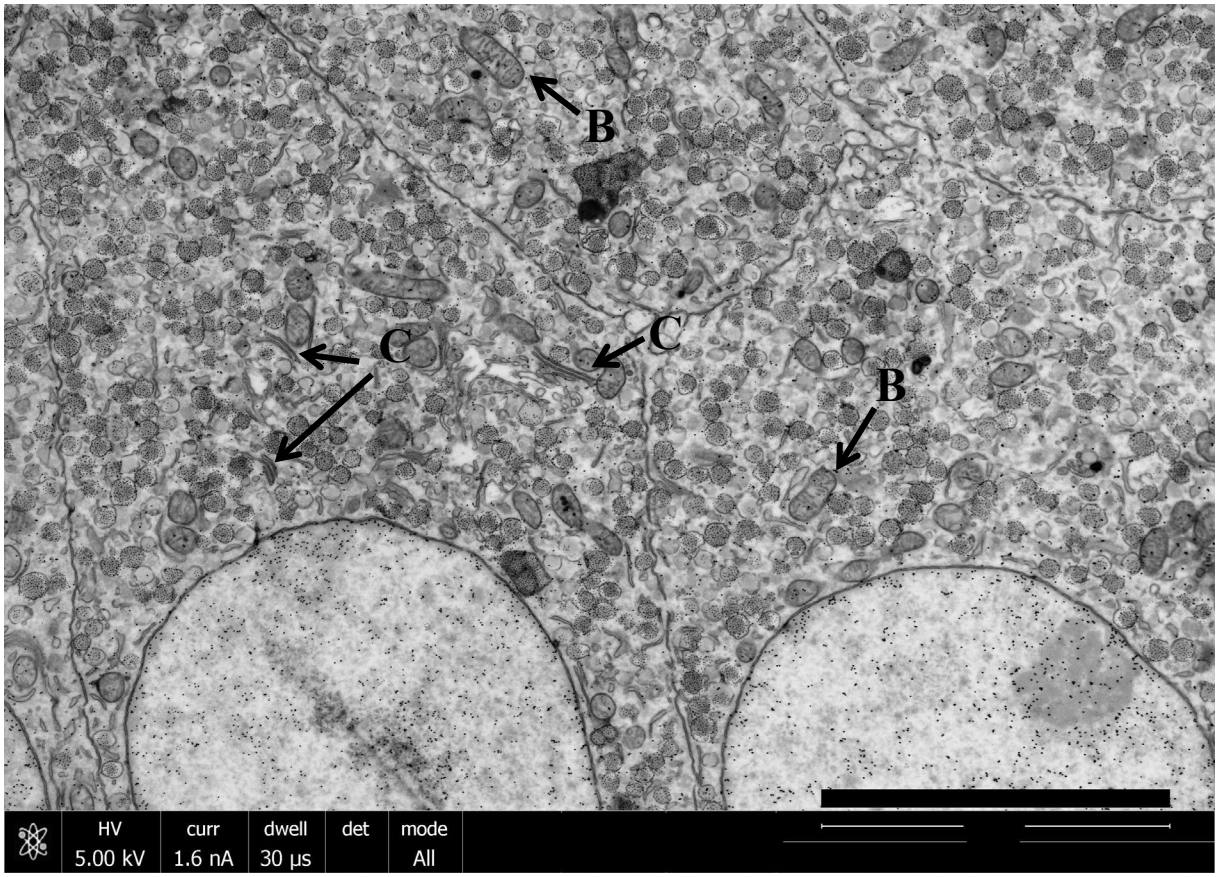
5. Look at the image on the next page. This has been recorded with an SEM.
(a) If you compare the acquisition parameters from this image (listed in the black bar) with the parameters listed in the methods section of the Ravelli et al. paper, how can you tell this is SEM and the other is TEM?

(b) Estimate the acquisition time for this one SEM image.

(c) What organelles are labelled with respectively B and C?

(d) What is your estimate for the size of the scalebar?

(e) In the sample, insulin granules have been labelled with quantum dots. How is this apparent in the image? And what would be the purpose of the labelling?


B
C
C
B
HV
5.00 kV
curr
1.6 nA
dwell
30 µs
det
mode
All