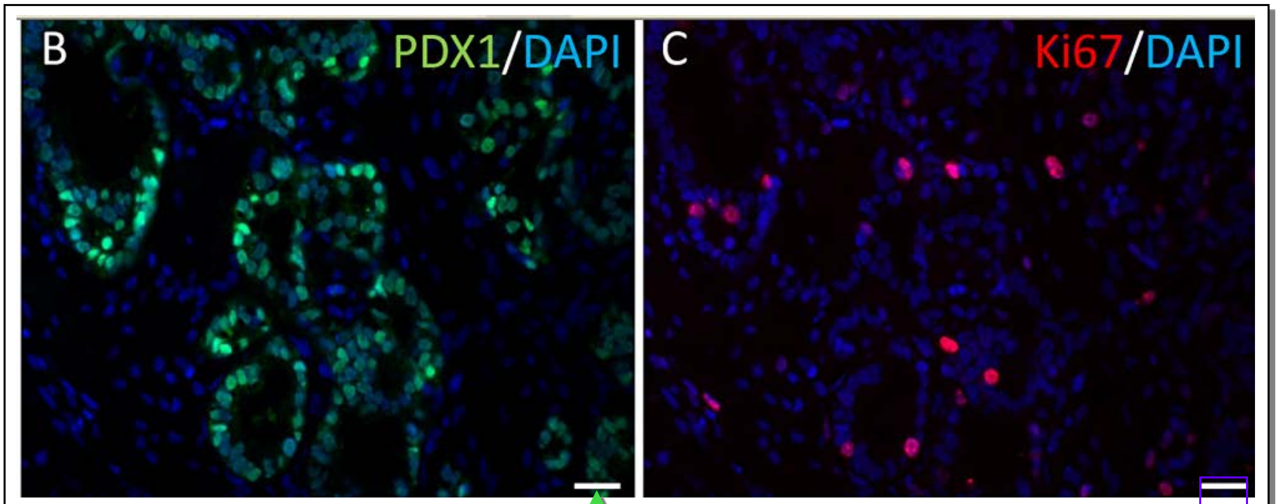
Calculating **Magnifications** using the Scale Bar

EXAMPLE of completed assignment, working with images found online

Image made using
↓ Fluorescence
Name: Doctor V.

1 ***Beta cells** in Islets of a developing pancreas*



6.3 mm

2 **Scale bar width 25 μm**

3 Actual size: about 6.3 mm = 6300 μm

4 $\frac{6300 \ \mu m}{25 \ \mu m} = 252 \rightarrow$

5 Pictures were magnified about **250** times.

6 **Brief description**. Inside the Islets of Langerhans, inside the pancreas, are the beta cells that release insulin into the bloodstream. Sometimes the number of beta cells can increase, as in cases of obesity. Sometimes, as in Type I diabetes, these beta cells are attacked by the body's own immune system. In Type II diabetes other factors contribute to making the beta cells dysfunctional.

(↑Science still in progress)

In this image, the aqua fluorescent **PDX1** shows, on the left, many beta cells that are "progenitor cells," which are early descendants of stem cells. On the right, the pink **Ki67** shows where cells are somewhere in the cell-dividing process. (The blue **DAPI** just marks DNA in nuclei of cells in the background)

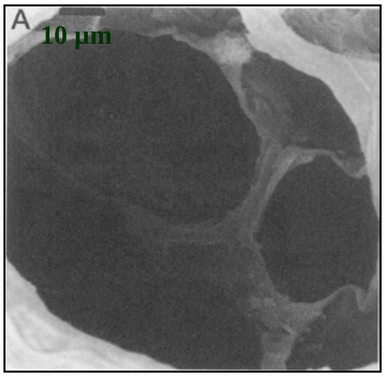
7 Image source: Raphael Scharffmann, XiangweiXiao, Harry Heimburger, Jacques Mallet, Phillippe Ravassard, "Beta Cells within single human Islets Originate from Multiple Progenitors," PLoS October 29, 2008
http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0003559

▶ NOTE: I found this example by searching the internet for: "beta cells scale bar" and then clicking on Images

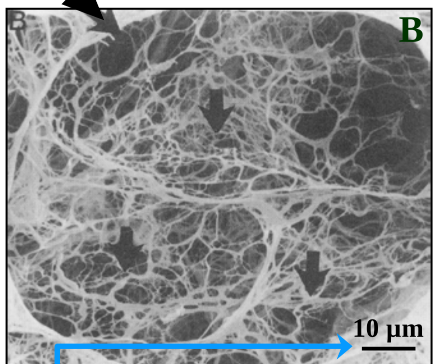
Another example of a completed assignment

1 **Collagen Fibers** in the alveoli of a lung
(Elastin fibers over here)

Image made using
Electron Microscopy
Name: Doctor V.

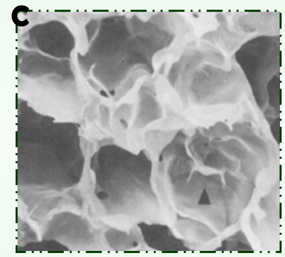


Detail from **Normal Human Lung**
Parts of alveoli seen (darkly) **from above**



Collagen in alveoli after elastin & cells were removed.

and on a smaller scale



Elastin in different **alveoli.** This time it's after collagen & cells were removed

2 **Scale bar says 10 µm**

3 Actual size: —— about 7 mm = 7,000 µm

4 $\frac{7000\ \cancel{\mu m}}{10\ \cancel{\mu m}} = 700$

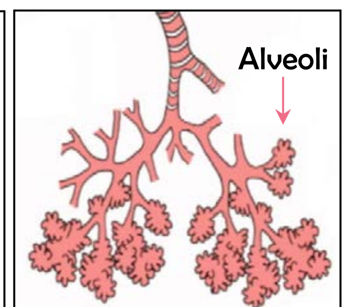
5 Picture **B** was magnified **700** times.

6 **Brief description**. These photographs were made to help understand the structure of the lung's **alveoli**. Alveoli are the little sacs in the lungs in whose walls red blood cells release CO_2 molecules and pick up O_2

To make image **B**, the scientists removed all the cells in the alveoli. They also removed the stretchable protein **elastin**, which is part of alveolar wall. They did this by submerging the tissue in a *lye* bath (sodium hydroxide, NaOH). Historically this has been used to remove hairs and elastin from animal skins in order to make them into leather for drums, bags, shoes, etc.

This process of "controlled alkali digestion," as the scientists call it, leaves behind the collagen fibers. Better yet, it "preserves the collagen frame-work in its natural location," showing just where collagen fibers are in the actual alveolar structures.

(The other pictures are just for the sake of comparison. The 3rd one, C, shows the result of placing lung tissue in an acid bath. This time, it was the cells & collagen that were removed, and the elastin that remained—lots of elastin.)



O_2: oxygen molecule
CO_2: carbon dioxide

↓ Leather shoe: *cow skin, with cells & elastin removed, collagen left over.*
Cow skin, like human skin, has more collagen than alveoli do

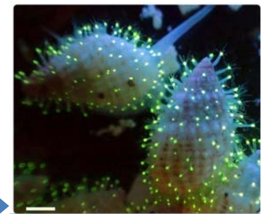
7 Source Finlay et al, "Elastin and Collagen Remodeling in Emphysema" *American J.Pathology* 1996
First two images from Figure 2, view from ducts. Last figure, elastin, from Figure 1, lower magnification

NOTE: I found this study by searching for "**ALVEOLI ELASTIN SCALE BAR**."

Source of shoe: Victoria & Albert Museum, UK. Shoe is probably pure leather, except for a few nails, etc.

Assignment #1, in detail

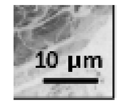
A. Search the internet for examples of magnified objects with scale bars . Choose an object that interests you.
Suggestions for places to look are on pages 4 and 5.
Go to Blackboard and see if anyone else has chosen the object first. If not you may "claim" it for your project. List your name, or initials, plus the object.



0.5 mm

B. Post the completed assignment on the Discussion Board.

1) Give us a title for the object you chose *and* your name and how the pictures was made
(with a light microscope? or electron microscope? or with fluorescence?)
2) What is the microscopic width of the scale bar in the picture?
That should be the number above or near the scale bar→
or mentioned in the caption beneath the picture (usually called **Fig.1, 2** …etc.)



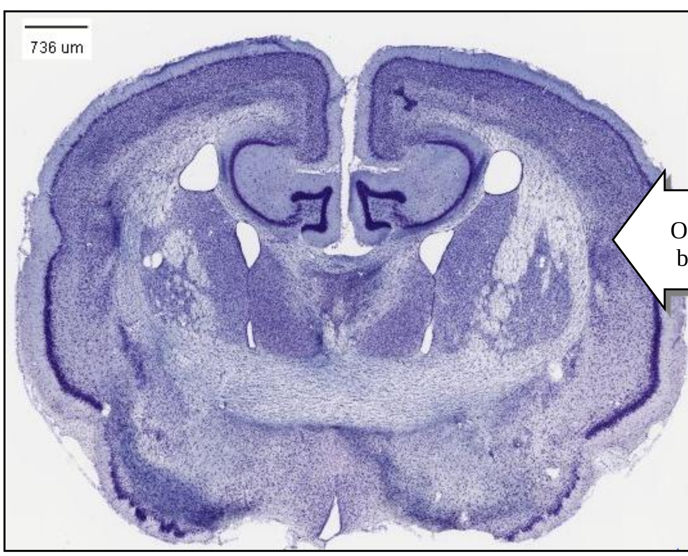
3) What is the actual width of the bar on the page? You can determine this by printing out the picture and measuring it with a ruler, or by using the method discussed on page **4**.
4) Set up the calculation:
Divide actual size of bar on the page by the size that scale bar is said to be (the smaller size)
5) What is the result of calculation? (How much was object magnified to make it the size that we see?)
6) Give a brief description (100 words or so) of what we see in the picture
(for example: define terms, give background, or basic principles)
7) Give us the source of the image of the object.

Another Example of a magnification calculations

This is a cross-section of a Marsupial brain. Image made using a light microscope

2) Original scale bar **736 micrometers** 3) Scale bar that I made: **8.0 mm** = 8000 µm

Here's a slice of brain from a small marsupial, a sort of opossum-mouse
Source: BrainMaps.org



One of many opossum brain cross-sections

4) Calculations 8000 microns / 736 microns = 10.87
5) Brain cross-section was magnified about 11 times,
Source: http://www.brainmaps.org/ An online brain atlas, covering a variety of animals

Source of top image, fluorescent Red Sea hydroids, living on sea snails, under UV light: VN Ivanenko . Phys.org

In case you're not familiar with measuring techniques, here are two ways to do it

Measuring the scale bar

Two methods, **a** & **b**

In the obese mouse (β), there are

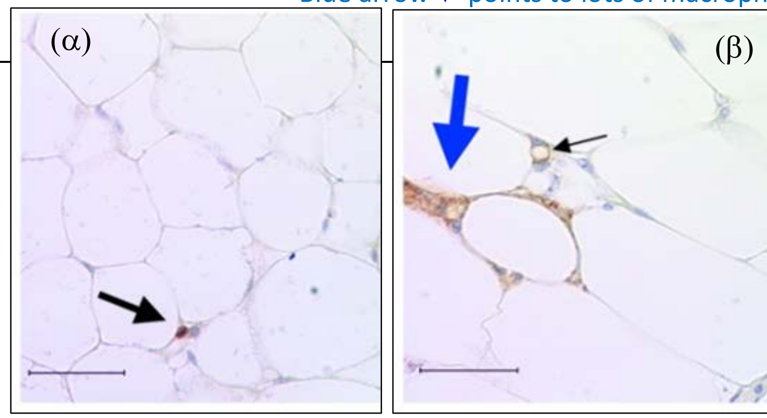
more macrophages ▶

among the adipocytes (fat cells)

than in lean mouse (α) →

LEAN mouse OBESE mouse

Blue arrow ▼ points to lots of macrophages



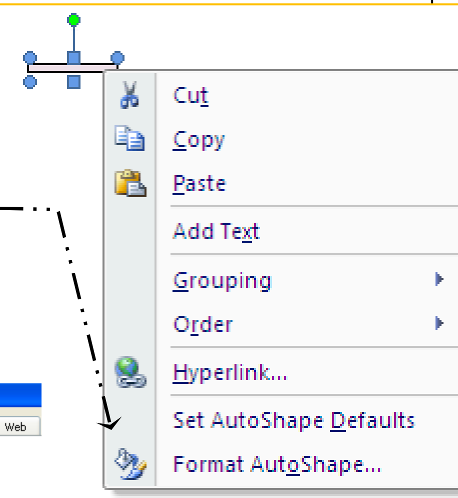
Source: Weisberg, Ferrante, JCI 2003

↑ 40 μm, **it says in source**

a 1. Find a picture that you like and download a copy of it. Then transfer the copied picture → onto a **document page.**

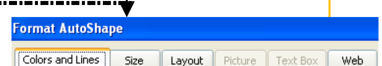
2. Now insert a RECTANGLE onto the page. ▲
Make the rectangle the <u>same width</u> as the scale bar.

3. Then right-click on the rectangle. A set of options appears→



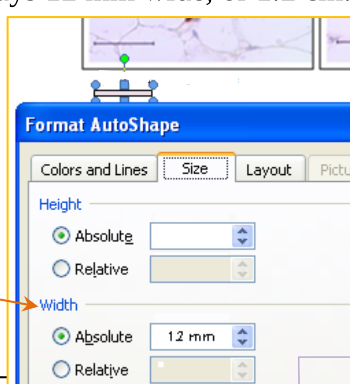
4. Click on **Format AutoShape**
or **More Layout Options** (it may depend on the computer)

5. A Format box appears.
On the band across the top, click on <u>Size</u>



6. A box appears showing the width of the rectangle (which is the same width as the scale bar).
In this case it says 12 mm wide, or 1.2 cm.

Width



← **12 mm**

The scale bar *says* 40 μm.
But here it is 12 mm wide.

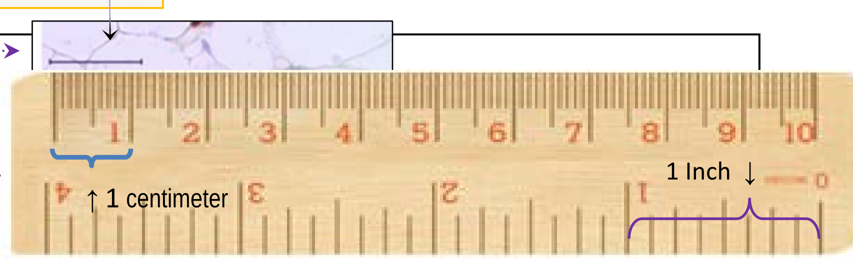
12 mm = 12,000 μm.
Divide by 40 μm.

→ Picture must have been magnified **300 times**

Or …

b …or you could just print the picture, & then measure the scale bar with a ruler

4 inches ≈ 10 cm →



Examples of Microscopic Objects that you might choose to look into

Possible objects include:

Microscopic parts of the mammalian body

(cells or parts of cells or layers: arteries, axons, capillaries, collagen, filopodia, mitochondria, neutrophil nets, lymphatic vessels, lysosomes, desmosomes, parts of ears, eyes, rods, cones, skin, intestines, brain, spinal cord, liver, spleen, pancreas parts of bones (osteons, osteoblasts, osteoclasts) kidney tubules, or glomeruli, heart [capillaries, myocytes] etc, etc, etc.

Microscopic parts from animals. Feathers, fur, fossils. Insects →

Fungi, slime molds or water molds, algae (diatoms, etc.) →→↓

Bacteria and Viruses and other Pathogens

Parts of flowers, leaves, plants, trees, seedlings, moss.

Refuse (paper, decaying weeds …)

Curculio ↑ (weevil)

←Navicula (diatom)

You might tell the search engine, for example, “filopodia, scale bar” and maybe “electron” or “fluorescence,” depending on what sort of picture you want

Sites you may wish to consult:

A guide to histology websites: http://www.siumed.edu/anatomy/histolinks.htm

One beautiful site: The Micropolitan Museum.

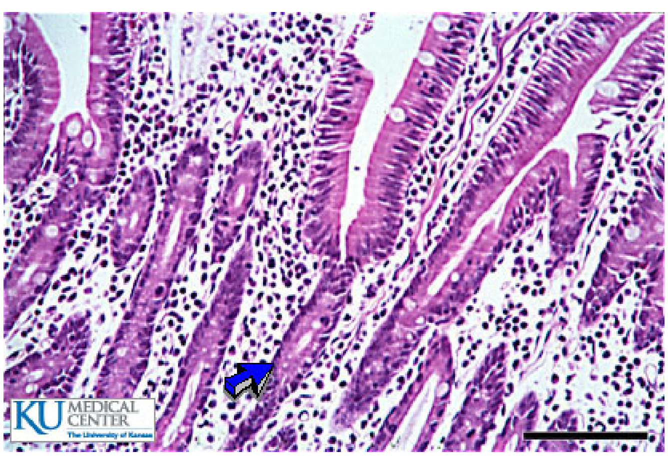
http://www.microscopyuk.org.uk/micropolitan/index.html

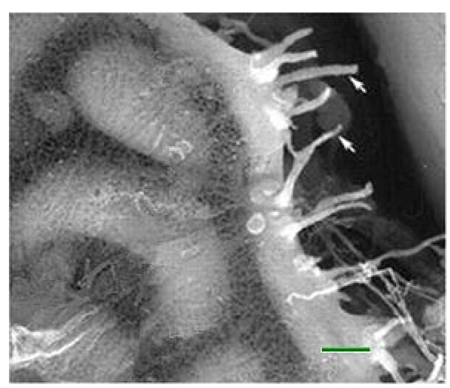
http://www.ou.edu/research/electron/www-vl/image.shtml

Problem: often there are no scale bars

A source of light-microscope images that I like is Kansas University Medical School. There’s a part called JayDoc’s Histoweb.

http://www.kumc.edu/instruction/medicine/anatomy/histoweb/nervous/nervous.htm





Filopodia (part of angiogenesis) ↑ UCSF

Electron or Fluorescence Microscopy

Another way to find microscopic pictures is to just google the words "fluorescence gallery" or SEM gallery or TEM gallery (One difficulty is that there are people named Tem who have put up galleries of images that have nothing to do with microscopy.)

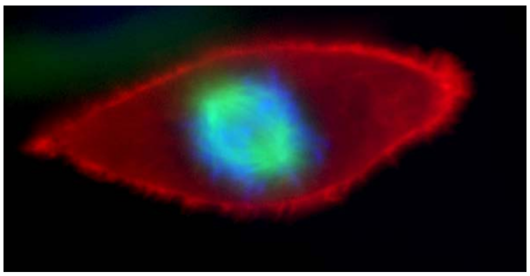
SEM & TEM are both kinds of electron microscopes; SEM images, seen below, are more "3-D"

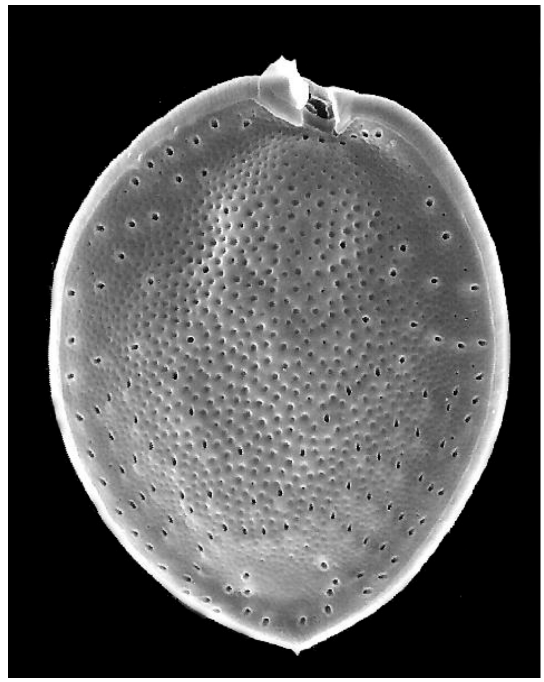
(Another difficulty: many of the images do not have scale bars)

Here are some examples:

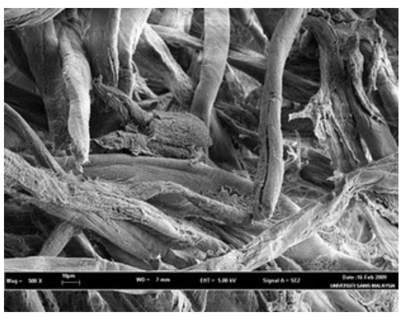
http://www.itg.uiuc.edu/exhibits/gallery/pages/image-18.htm

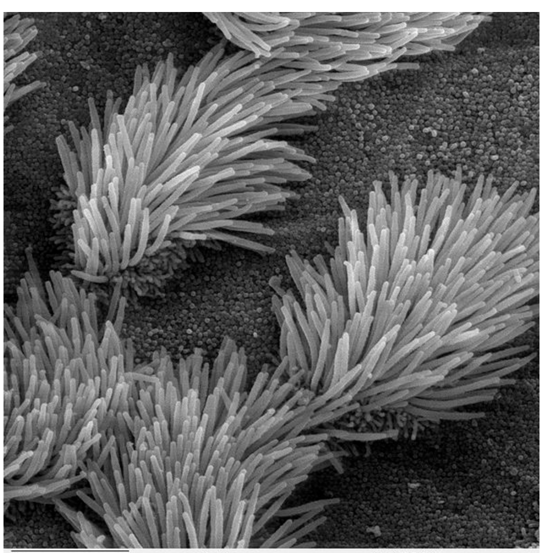
http://www.mnh.si.edu/highlight/sem/dinoflagellates.html





http://adrianchek.blogspot.com/2009/02/sem-of-filter-paper-degradation.html



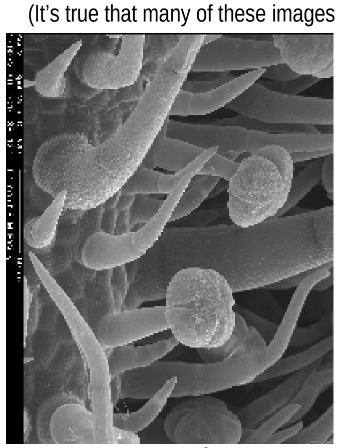


←5 μm Cilia in trachea of lungs

Dartmouth Electron Microscope Facility .

http://www.dartmouth.edu/~emlab/gallery/

(It's true that many of these images don't have scale bars, but many do)



← ~50 μm Tomato leaves