Week 1: The Size of Things

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1 Introduction

A first step in understanding new scientific concepts is getting a feel for the scales involved in the problem. The size of the organism, the time it takes for a process to occur, the amount of energy consumed, are all questions that need to be answered before a biological organism can be studied in detail. This week, we will concentrate on the "Size of Things". We will look at the size of some of the most studied biological organisms and use microscopy techniques to visualize them. For a good introduction on microscopy and spatial calibration, please check out the relevant handout in the APh162 website:
http://www.rpgroup.caltech.edu/courses/aph162/2007/Protocols/microscopy.pdf

1.1 Goals for this week's lab:

The goal of this lab is to look at some of the most studied biological organisms and take their pictures using various microscopy techniques. The organisms you will look at are briefly described in the following section. You will post these pictures on your websites but make sure there is a scale bar on each. It is not at all informative when a picture is shown, whether in a paper or in a presentation, and the scale bar is missing. In fact, this is a good rule to keep in mind: **Always show the scale bar that comes with your picture!** In order to be able to report the scale bar, you will first need to spatially calibrate your microscopes.

2 Spatial Calibration

In order to be able to tell what the size of the organism that you are looking under the microscope is, you will first need to spatially calibrate the microscope for the magnifications that you will be using. So, as a first step, you will use bright-field microscopy to calibrate different magnifications on the microscope, in order to be able to relate what you see under the microscope to physical distances. You will image a lithographic graticule with $10\mu m$ markers at different magnifications and produce a "calibration curve". In other words, you will look at the graticule and take its picture at different magnifications. You will then pick a distance of the imaged graticule (that will be the actual physical distance) and find out how many pixels in your

image it corresponds to (that will be the imaged distance). In this way, you will make a graph of the pixels/mm (y axis) vs. the magnification (x axis). Of course, this graph is applicable only to the microscope you were using. Every microscope has its own calibration curve. Therefore, if you use a different microscope, you will have to create a new calibration curve. For this reason, after you have calibrated at least one microscope, feel free to exchange calibration curves with the other groups. Make sure you know which calibration curve corresponds to which microscope!

3 The Biological Organisms

Below we give a list and a brief description of the biological organisms we will look under the microscope in order to get a feel for their size.

***Escherichia coli* (*E. coli*):** One of the most studied prokaryotic organisms is *E. coli*. You can consider this as the unit of measurement to which all other prokaryotic organisms can be compared. It was discovered by Theodor Escherich, a German pediatrician and bacteriologist. *E. coli* is one of the main species of bacteria that live in the lower intestines of mammals, known as gut flora. Specimens have also been located on the edge of hot springs. According to US Department of Health and Human Services Centers for Disease Control and Prevention, the E. coli strain O157:H7, one of hundreds of strains of the bacterium E. coli, causes illness in humans. Presence in surface water is a common indicator of fecal contamination. It belongs among the Enterobacteriaceae, and is commonly used as a model organism for bacteria in general. One of the root words of the family's scientific name, "enteric", refers to the intestine, and is often used synonymously with "fecal" (Wikipedia).

For this part of the lab, you will take snapshots of *E. coli*cells at different magnifications. When you post these pictures on your website, do not forget to show the scale bar!

***Saccharomyces cerevisiae* (*S. cerevisiae*):** *S. cerevisiae* is a species of budding yeast. It is perhaps the most important yeast owing to its use since ancient times in baking and brewing. It is believed that it was originally isolated from the skins of grapes (one can see the yeast as a component of the thin white film on the skins of some dark-colored fruits such as plums; it exists among the waxes of the cuticle). It is one of the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model prokaryote. It is the microorganism behind the most common type of fermentation. It reproduces by a division process known as budding. It is useful in studying the cell cycle because it is easy to culture, but, as a eukaryote, it shares the complex internal cell structure of plants and animals. *S. cerevisiae* was the first eukaryotic genome that was completely sequenced. The genome is composed of about 13,000,000 base pairs and 6,275 genes, although only about 5,800 of these are believed to be true functional genes. It is estimated that yeast shares about 23% of its genome with that of humans. "Saccharomyces" derives from Greek, and means "sugar mold". "Cerevisiae" comes from Latin, and means "of beer" (Wikipedia).

Again, for this part of the lab, take several snapshots of the yeast cells at different magnifications and report your pictures with the relevant scale bars.

***Caenorhabditis elegans* (*C. elegans*):** *C. elegans* is a free-living nematode (roundworm), which lives in temperate soil environments. Research into the molecular and developmental biology of C. elegans was begun in 1974 by Sydney Brenner [1] and it has since been used extensively as a model organism. *C. elegans* is used as a model organism for a variety of reasons, including economy, and the ease of maintaining a population in the laboratory. Worms can be frozen and subsequently thawed and remain viable, thus ensuring easy long-term storage of different worm strains. Because the complete cell lineage of the species has been determined, *C. elegans* has proven especially useful for studying cellular differentiation. From a research perspective, *C. elegans* has the advantage of being a multicellular eukaryotic organism which is simple enough to be studied in great detail (Wikipedia).

Obtain pictures of this organism and report on your websites.

***Dictyostelium*:** *Dictyostelium* is a cellular slime mold and belongs to the rank of myxomycetes. It is a pseudoplasmodium composed of separate cells (Carolina). When food (normally bacteria) is readily available the cells take the form of individual amoebae, which feed and divide normally. However, when the food supply is exhausted, they aggregate to form a multicellular assembly, called a pseudoplasmodium or slug (not to be confused with slug the animal). The slug has a definite anterior and posterior, responds to light and temperature gradients, and has the ability to migrate. Under the correct circumstances the slug matures forming a fruiting body with a stalk supporting one or more balls of spores. These spores are inactive cells protected by resistant cell walls, and become new amoebae once food is available (Wikipedia).

Obtain snapshots of this organism and include in your website. Do not forget the scale bar!

***Didymium nigripes* :** *Didymium nigripes* , just like *Dictyostelium*, is another type of slime mold culture and also belongs to the rank of myxomycetes. It exhibits protoplasmic streaming and forms sporangia readily (Carolina).

RAW 264.7 macrophages: RAW 264.7 is a mouse leukaemic monocyte macrophage cell line. The line was established from the ascites of a tumor induced in a male mouse by intraperitoneal injection of Abselon Leukaemia Virus (A-MuLV). Cells have receptors for immunoglobulin and produce lysozyme . They are used in metabolic, inflammation and apoptosis studies (http://www.abcam.com/index.html?datasheet=7187).

A sample will be provided to you in a culture chamber. If you get the change, observe the cell when they are attached on a surface and when they are floating in solution. What are the physiological differences (size, shape, etc.) that you can observe between the two states? Take some snapshots and report them in your websites with the scale bars.

***Drosophila* eye:** *Drosophila* is arguably the most versatile and one of the most powerful eukaryotic genetic model systems. Flies are easy to maintain and breed in large numbers in the lab and have a generation time of 10 days (at 25 C). The genome is relatively compact (1.7 x 108 bp and has been completely sequenced - the annotated version has been freely available on the web since March 2000. *Drosophila* has a typical insect compound eye. Each eye is composed of several hundred simple units called ommatidia arranged in an extremely regular array (http://www.ucl.ac.uk/ ucbhhks/FlyI.htm). It is an example of a cellular crystal (hexagonally closed packed).

Take pictures of the eye and report on your website with the scale bar. If you get the change, take the Fourier transform (absolute value) in order to see the hexagonal structure.

***Stentor polymorphus*:** Stentor protozoa are single-celled animals that grow to a length of 1.5 to 2 millimeters, much larger than many of their fellow multi-cellular aquatic organisms. Often, stentors will attach the lower portion of their pod to debris and assume a trumpet-like shape. The circle of tiny cilia surrounding the trumpet rim beat continuously and serve to create localized convection currents in the water to draw smaller organisms into the rim or mouth (cytostome). Stentors vary in color, depending upon their diet, but they can appear green, blue, or reddish-yellow.
(http://micro.magnet.fsu.edu/primer/techniques/hoffmangallery/stentor.html).

The trumpet-like shape is what gave this organism its name. Stentor was a figure in the Greek mythology. He was a herald of the Greek forces during the Trojan War, made famous by his loud voice (the adjective "stentorian" means loud-voiced). He died after he was defeated by Hermes in a shouting contest.

Again, take some pictures to add to your website, together with the scale bars!

B-cells: B cells are lymphocytes that play a large role in the humoral immune response as opposed to the cell-mediated immune response that is governed by T cells. The abbreviation "B" comes from bursa of Fabricius that is an organ in birds in which avian B cells mature. The principal function of B cells is to make antibodies against soluble antigens. B cells are an essential component of the adaptive immune system (Wikipedia).

Obtain images of these cells to include in your website.

Bovine pulmonary cells: You will be looking at bovine pulmonary artery endothelial cells (BPAEC). For slide 1, the mitochondria are stained with MitoTracker Red CMXRos, F-actin is labelled with BODIPY FL phallacidin, and the nuclei are labelled with DAPI (Invitrogen, Catalog Number - F-14780). For, slide 2, Texas Red-X phalloidin is labelling the F-actin, antibovine -tubulin mouse monoclonal 236-10501 in conjunction with BODIPY FL goat antimouse IgG antibody is labelling microtubules, and DAPI is labelling the nucleus (Invitrogen, Catalog Number - F-14781). For the data sheet to these slides, take a look at:
http://probes.invitrogen.com/media/pis/mp14780.pdf

Since the different organelles are labelled with different dyes, this will be an exercise in fluorescence microscopy. Using 3 different microscope filters (FITC, TRITC, and DAPI), you will visualize the actin, mitochondria and nucleus, respectively. Then, use a software of your choice to combine the 3 images into one single colored image of the entire cell.