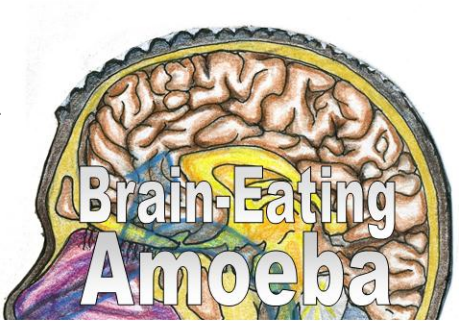
Brain-Eating Amoeba

An Interdisciplinary Research Simulation Case Study



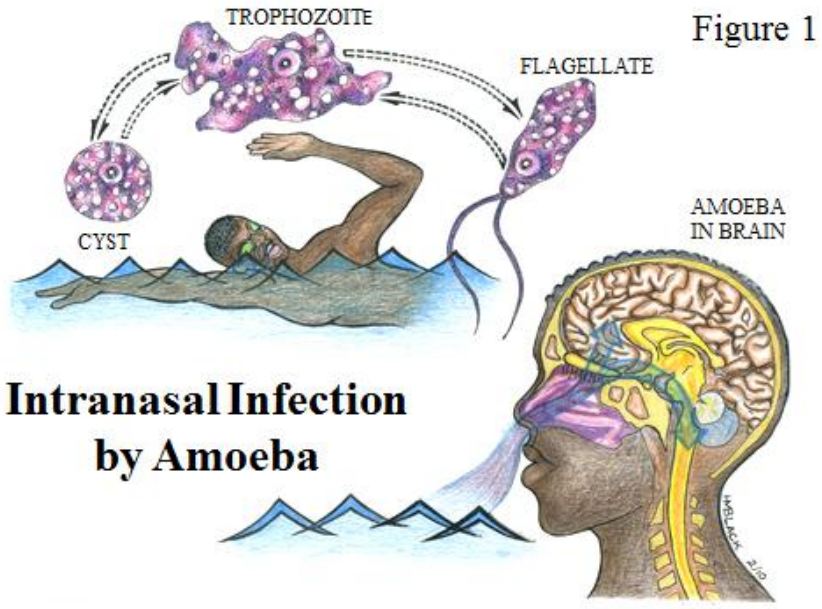
A. Brain-Eating Amoeba:
The day after diving, swimming, and having fun in a Florida river, a 16 year old girl, developed a severe headache, vomiting, and a fever of 102^0F (degrees Fahrenheit). She was taken to the hospital and sent home with pain medication and an antibiotic. The next morning, her fever rose to 104^0F, she became delusional and was rushed back to the hospital where she was diagnosed with a *Naegleria fowleri* (*N. fowleri)* infection. Even the best hospital care was not good enough to stop the ravaging infection and sadly, the girl died two days later.

N. fowleri is an amoeba, a single celled organism that can be found living free, mostly in aquatic environments. Most species of amoebas are harmless to humans, but *N. fowleri* is not harmless and can be a deadly pathogen (disease causing organism). *N. fowleri* is acquired when contaminated water enters the nose, but not the mouth. The pathology of *N. fowleri* infections has been investigated by studying infections in mice. This amoeba introduced into the nostril of a mouse migrates, by way of the olfactory nerve, to the brain. Mice die within several days of infection. *N. fowleri* causes a disease in humans called primary amebic meningoencephalitis (PAM). PAM causes swelling in the brain, destroys brain tissue, and is almost always fatal. This rare disease was first discovered in the 1960s, and since has been reported in over 15 countries. In the United States, there have been 128 reported cases since 1962 and only one person has survived the infection.

The Research – Part I: *Imagine you are a research scientist interested in sampling environmental sites to warn people of the possibility of contracting PAM. The first thing any scientist must do before starting a research project is to collect background information. The background information you need to get started is provided in the following two sections, B and C.*

B. Life-Cycle of *N. fowleri:* The life-cycle of *N. fowleri* includes a dormant cyst, an amoeboid feeding stage (trophozoite), and a rapidly swimming flagellate. When freshwater conditions are not favorable for growth, *N. fowleri* exist in the cyst form. Trophozoites are thermophiles (heat-loving organisms) and survive and reproduce most efficiently at warm temperatures. When food resources are scarce, the trophozoite enters the flagellate form, which swims to another location searching for more food sources. Once food is found, the flagellate turns back into the trophozoite stage. Only the trophozoite stage is capable of infecting a human. Even in warm or thermally polluted waters, *N. fowleri* is always found in low numbers in the environment and *N. fowleri* infections are extremely rare.

Figure 1



C. Warm Freshwater: *N. fowleri* infections have been linked to warm freshwater sources. These can be naturally occurring warm waters or waters that have been artificially increased by thermal pollution. Thermal pollution is the increase or decrease of water temperature caused by human activity. Increased water temperature can drastically change the ecosystem by decreasing the levels of oxygen in the water and impacting organisms' life cycles or food sources. *N. fowleri* grows well between 35^0C and 46^0C, and grows fastest at 42^0C. Most other amoeboid species do not survive at temperatures at or above 42^0C. Water temperatures of 50^0C will kill all life-cycle forms of the *N. fowleri* amoeba. *N. fowleri* exist in the cyst stage at temperatures less than 26.7^0C. Temperatures below 20^0C inhibit reproduction and temperatures below 10^0C destroy the organism. *N. fowleri* cannot grow in salt water. Chlorine kills all life-cycle stages of *N. fowleri* and is the most effective way to disinfect swimming pools.

The Research – Part II: *Now that you have some background information on N. fowleri, you can begin your scientific investigation consisting of sampling environmental sites for N. fowleri. You are interested in looking at bodies of water in your general geographic location. First you search online for the range of water temperatures in five different sites near you. Let's suppose the data you found is presented in Table 1. Some of these sites have been thermally polluted. The first thing you notice, as a scientist, is that this data is not acceptable, because scientists use the centigrade scale or Celsius (^{o}C), and the information online was recorded in degrees Fahrenheit (^{o}F).*

Environmental Sites	^{o}F Range
Site 1	61^o-76^o
Site 2	92^o-113^o
Site 3	124^o-136^o
Site 4	35^o-68^o
Site 5	75^o-107^o

Table 1

Research Activities:

1. Draw a simple X-Y graph plotting degrees Fahrenheit versus degrees Celsius. Using the formulas below, convert the following temperatures from degrees ^{o}F to ^{o}C: 32^0F, 120^0F and 212^0F. Draw a line through the points on the graph.

$$T_c = \frac{5}{9} \times (T_f - 32) \qquad T_f = (\frac{9}{5} \times T_c) + 32$$

T_c = temperature in Celsius T_f = temperature in Fahrenheit

2. Using the graph you created, extrapolate (determine) the lowest and highest temperature from each site in Table 1 on your graph, and record these temperatures. Suppose you are only interested in testing sites that may contain the pathogenic trophozoite form of *N. fowleri*, which of these sites would you test, or not test, and why (be specific for each site)?

3. You are also interested in identifying three more potential environmental sites from a different geographic area that might contain the trophozoite form of *N. fowleri*. Describe three different natural geographic locations or thermally polluted sites you would sample. An example of a description that you might make is "a shallow river in southern Florida."

4. Describe the method, or how you would collect samples from your environmental sites. Include in your answer how many samples you would take from each site and why.

The Research – Part III: *As the competent research scientist that you are, you realize that just collecting samples and determining the water temperatures in ^{o}C for environmental sites near you, is not enough to warn the public of a potential danger. In fact there are many thermally polluted sites and naturally occurring warm bodies of water that do not contain N. fowleri. You realize that you need an experimental procedure that will allow you to specifically identify the amoebas in your samples taken from the different environmental sites. You also realize that there are dozens of other free living amoebas found in the environment and you must be able to tell the difference between these amoebas and pathogenic N. fowleri. Suppose after searching the scientific literature you discovered the following background information that is provided in sections D and E.*

D. Indirect Immunofluorescence: Indirect immunofluorescence is one technique that will let you identify *N. fowleri* and differentiate it from other species of amoebas. *N. fowleri* can be grown (cultured) in a research laboratory. *N. fowleri* cells can grow on petri dishes that contain bacteria, which they eat. Cultured *N. fowleri* can be injected into the blood stream of a rabbit, causing the rabbit's immune system to produce an immune response by generating antibodies. These anti-*N. fowleri* rabbit antibodies (made in the rabbit) can be isolated from the rabbit's blood. In a test tube, anti-*N. fowleri* antibodies can bind ***specifically*** to *N. fowleri* amoebas and will allow you to detect the amoebas using various types of laboratory equipment. It should be noted that these specific anti-*N. fowleri* primary rabbit antibodies bind best to *N. flowleri*, and bind to a much less degree or not at all to other types (species) of amoebas.

Now that *N. fowleri* is bound to the rabbit antibody (primary antibody), the technique calls for adding to your test tube a secondary antibody. This secondary antibody is able to bind to any rabbit antibodies, as demonstrated in Figure 4. Also, this secondary antibody has a fluorescent dye or marker called fluorescein (FITC). FITC will produce a green glowing light (it fluoresces) when it is exposed to the appropriate light source. The entire process of mixing primary antibodies with FITC bound secondary antibodies is called indirect immunofluorescence.

Figure 5 is a fluorescent microscope image of many *N. fowleri* cells fluorescing after they have been mixed with anti-*N. fowleri* rabbit antibodies, FITC bound secondary antibodies, and exposed to the appropriate light source. *N. fowleri* cells were cultured on a petri dish with many bacteria (such as *E. coli)*. The amoebas then eat the bacteria and grow into a very dense population of cells, which you can see in figure 5.

Indirect Immunofluorescence

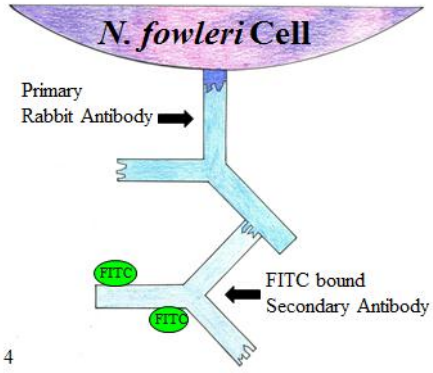


Figure 4

Fluorescent Microscope Image of *N. fowleri*

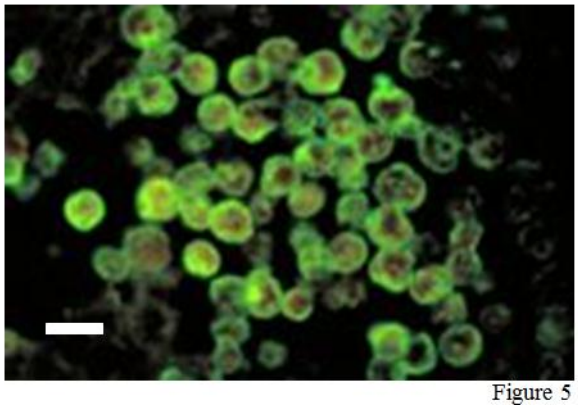


Figure 5

Research Activities:

5. The white line in the fluorescent microscope picture (Figure 5) of *N. fowleri* is 20 micrometers (μm) in length. What is the approximate range of sizes of the fluorescing *N. fowleri* cells in μm? What is the approximate range of sizes of these cells in mm and nm?

Note that: 1 millimeter (mm) = 10^{-3} meters (M), 1 micrometer (μm) = 10^{-6} M, 1 nanometer (nm) = 10^{-9} M

E. Flow Cytometer. Another scientifically advanced way of analyzing cells for size, (as you manually did in Figure 5) is to use a flow cytometer. Figure 6 is a picture of one type of flow cytometer. Figure 7 depicts how a flow cytometer works.

In the flow cytometer, cells flow in a liquid past a focused laser light beam (Figure 7). The degree the laser is scattered when it hits the cell determines the cell size. This scattering of the laser light is read by the detector as seen in Figure 7. If the cells have been stained with a fluorescent dye and the laser light source is the correct wavelength, then the fluorescent dye on the cells will glow. This glowing fluorescent light can also be read by the detector. In Figure 7, note that some cells in this illustration are fluorescing green, and some cells are not fluorescing (as illustrated by the black dots). A flow cytometer can measure thousands of cells per minute for cell size and fluorescent intensity and then graph this data on an X-Y axis graph on a computer screen using special software. These graphs are called cytograms. Each dot on a cytogram represents a single cell. Larger cells appear more vertical on the Y axis and cells that are brightly fluorescing appear more horizontal on the X axis.

Figure 6

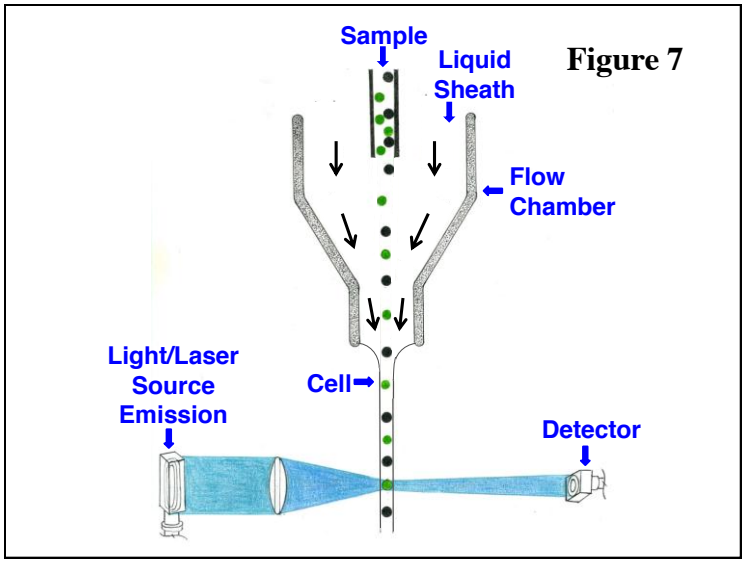


Figure 7

Research Activities

6. A control research experiment was set up to illustrate how fluorescing cells would graph differently from non-fluorescing cells. In Figure 7 there are ten fluorescing cells and ten non-fluorescing cells. The cell size and degree of fluorescence for each of these cells is given in Table 2 below. Create a cytogram from the cells in Figure 7 by drawing an X-Y graph, with fluorescence as the X axis, and size the Y axis. Then plot a dot on this graph for each of these twenty cells.

Table 2.

Flu. Cells	1	2	3	4	5	6	7	8	9	10
Cell Size µm	17	9	15	12	10	14	16	12	14	15
Level of Flu.	75	43	50	45	60	65	55	63	57	52
Non Flu. Cells	1	2	3	4	5	6	7	8	9	10
Cell Size µm	16	14	10	13	12	14	17	11	12	15
Level of Flu.	1.1	1.0	1.4	1.6	1.0	1.6	1.0	1.1	1.3	1.0

*Flu. in Table 2 represents "fluorescence."

The Research – Part IV: *Before you design your protocol and perform your experiments to test sites for N. fowleri, you must learn how to select the correct laser and detector to use in your flow cytometer. This is covered in sections F and G.*

F. Fluorescence: An interesting point about the flow cytometer is that in every experiment you must use a laser and a detector that works with your fluorescent dye (or simply called **marker**). To do this you must understand how fluorescence works. In general terms, fluorescence occurs in two steps. First, an electron in your fluorescence marker is excited into a more active state by the laser. When the electron relaxes back to its ground state, it emits glowing fluorescent light. Figure 8 demonstrates how fluorescence occurs in more detail along with the correct terminology needed to ultimately select a laser and detector. Specifically, it can be seen in Figure 8 that a laser light generates a specific excitation wavelength that will excite the fluorescent marker electron to a higher energy level (indicated by the straight blue line). Note, only a specific excitation wavelength in nanometers (nm) will excite a given marker. Next, in step 2, the electron relaxes (indicated by the straight green line) to its original ground state, and the resulting fluorescent light is generated at a specific emission wavelength in nm. The specific emission wavelength of light in nm determines the color of the light. Finally, a specific detector can only read a specific emission wavelength of fluorescing light.

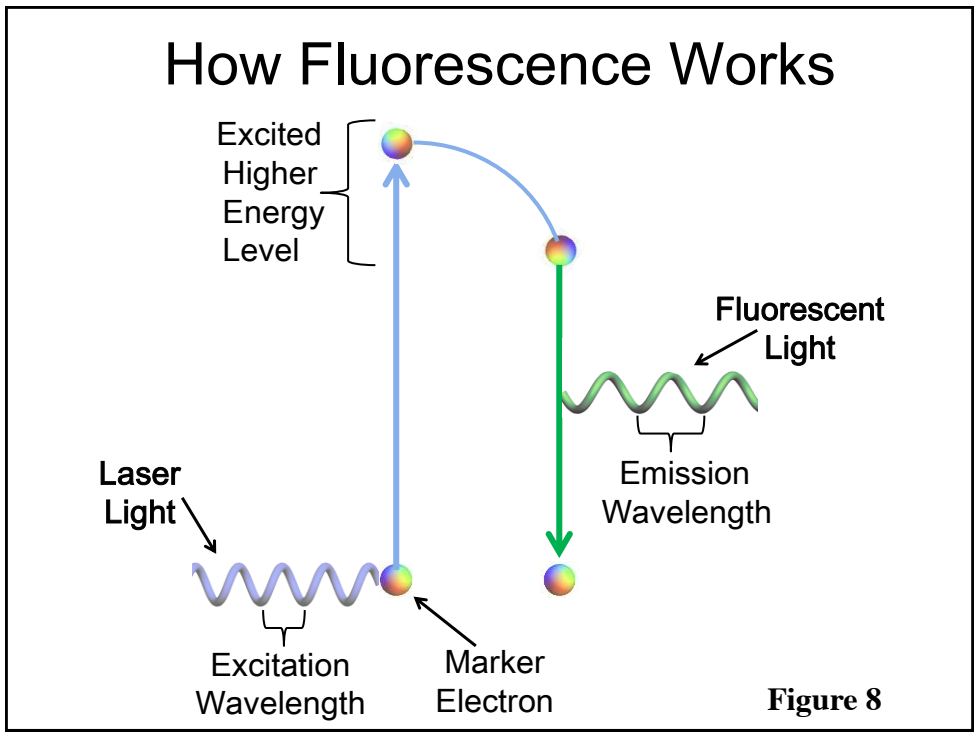
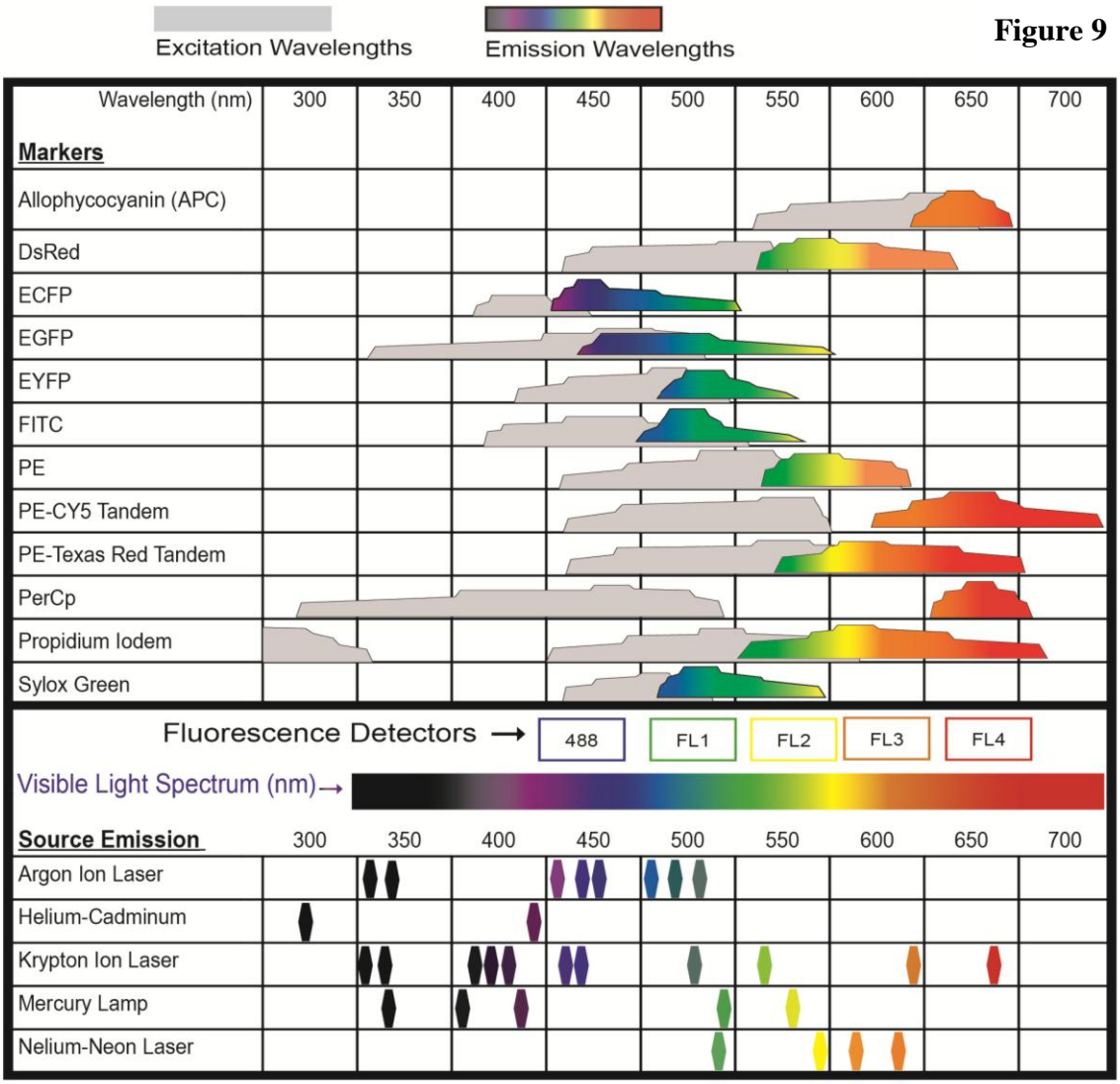


Figure 8

G. Laser and Detector Selection: Now you are ready to select a laser light source and a detector for your experiment. Figure 9 contains the excitation (solid gray) and emission (light spectrum) wavelengths of 12 fluorescent markers. The wavelengths required for excitation and emission of each marker is also depicted in this figure. The fluorescence detector boxes (i.e. 488, FL1, FL2, FL3 or FL4) are aligned in this figure with the specific wavelength in nm that the detector picks up and reads.

Figure 9



Research Activities:

7. Which **laser** would you choose for your experiment? Be sure to choose a laser/light with a source emission wavelength that would generate the best excitation of the marker you are using in your experiment with anti-*N fowleri* rabbit primary and FITC-secondary antibodies. Explain in detail why you made this choice.

8. Which **detector** would you select for your experiment and why? What wavelengths does this detector read?

9. Given the information in this case study, outline the simplest protocol that can quickly and easily survey for and identify *N. fowleri* in the environmental samples you have collected in the first part of this case study. Remember to add a control or two to your protocol. A control for this experiment refers to testing a control population of cells that you know are *N. fowleri*. This would be a positive control. A negative control would be a population of cells that you know does NOT contain *N. fowleri*. Using these two controls, you can compare your environmental samples to determine if *N. fowleri* is present or absent. This is done in order to make sure that your experimental protocol to identify *N. fowleri* does not mistakenly identify an entirely different amoeba or a closely related species of *N. fowleri*. *N. fowleri* is one of 45 identified species of *Naegleri*, such as *N. lovaniensis* and *N. guberi*, which are non-pathogenic.

The Research – Part V: *Your original research objective was to sample environmental sites to warn the public of the possible existence of pathogenic N. fowleri. In this case you have developed environmental sampling methods for this pathogen, experimental procedures and controls to identify N. fowleri, and configured your flow cytometer with the correct lasers and detectors. Now let's assume you have conducted these experiments and you are analyzing your data. Figure 10 represents the cytogram results from your control. Figure 11 represents the results from samples taken from two environmental sites (Site #2 and Site #5).*

Research Activities:

10. In this control experiment represented by Figure 10, thousands of *N. fowleri* and *N. lovaniensis* cell were mixed. Which cluster of cells (A or B) represents *N. fowleri*, and which cluster represents *N. lovaniensis?* Explain your answer.

11. In the cytogram B labeled Site #5 of Figure 11, what is your interpretation of the cluster of cells found primarily in Box 3? Do you think you have identified *N. fowleri* and why?

12. In the cytogram A labeled Site #2 of Figure 11, what is your interpretation of the cluster of cells in Box 1?

13. In the cytogram A labeled Site #2 of Figure 11, what is your interpretation of the cluster of cells in Box 2?

14. To confidently warn a community about a *N. fowleri* threat, you must be sure. What could you do to determine, definitively, if you have isolated pathogenic *N. fowleri* from the sample that gave the pattern in Site #5? What could you do with the cells that generated the cluster in cytogram site #2, Box 2 to collect further data to determine if you isolated a pathogenic amoeba or not.

Control Cytogram

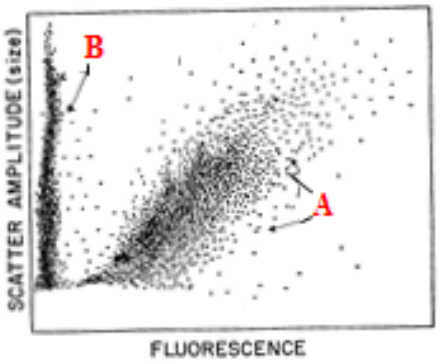


Figure 10

Cytograms from Environmental Sites

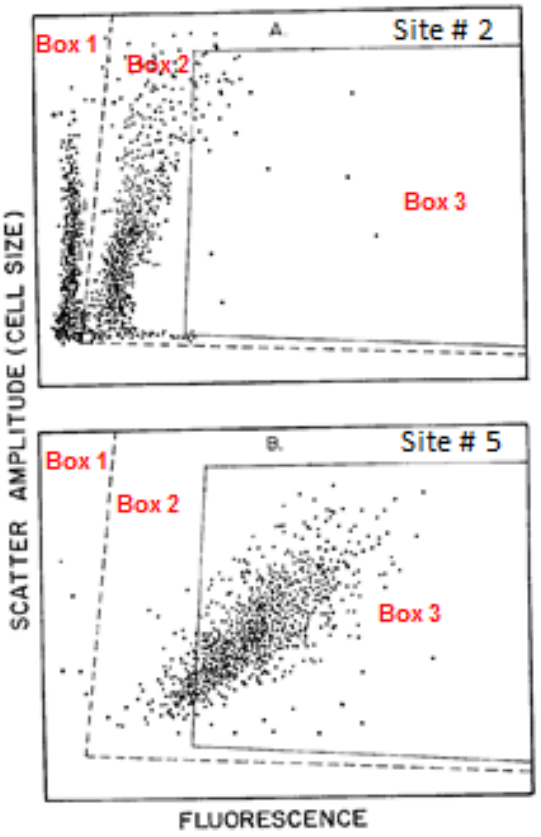


Figure 11

H. Interdisc iplinary Research: An academic discipline is a branch of knowledge that is taught, studied or researched. For example, biology, mathematics, chemistry and physics are well established examples of disciplines that are taught in high school. As a student progresses to the college or university level, these four basic academic disciplines branch into dozens of other disciplines or sub-disciplines. Each of these disciplines or sub-disciplines may represent different college courses and even different majors in college and graduate school.

In today's evolving world of science, many research problems do not lend themselves to be solved by one discipline; consequently, interdisciplinary research is required. Interdisciplinary research occurs where one or more disciplines are integrated to answer a single question. This case study, Brain Eating Amoeba, required an interdisciplinary approach to determine if *N. fowleri* is present in a body of water.

Research Activity:

15. For you to be an effective scientist and solve the research problem in this case study, you had to conduct background studies in specific disciplines. Each discipline in this case study was labeled by an alphabet (i.e. A through G). Your next assignment is to identify the disciplines you studied by placing the letter of that section (A through G) in the Table 3 next to the biology, chemistry and physics disciplines. Place only one alphabet per box. For the discipline of mathematics place a number (1 through 14) for the "Research Activities" that required Mathematics. Place only one number per box.

Disciplines & Descriptions	Sections A-G
Biology - Study of life sciences	
• Pathology – study and diagnosis of disease	
• Protozoology – study of single cell organisms	
• Environmental Science – relationships among organisms & environment	
• Immunology – study of the immune system	
• Cell Biology – study of cells (note "cyto-" is a prefix meaning cell)	
Chemistry – focuses on composition and properties of matter	
• Photochemistry – chemical reactions that emit or absorb light	
Physics – science of matter, energy, force and motion	
• Optics – science of light	
Mathematics – focuses on relationships among numbers	
• Algebra – uses symbols like letters to represent unknown numbers	
• Metric System – international system of units (not a discipline)	

Table 3

By Lycurgus L. Muldrow, Ph.D., Alicia Thomas, Harun Black, Mellissa Demetrikopolous, Ph.D., and Elisabeth H. Javazon, Ph.D.