Key: Yellow highlight = required component

To Catch a Microbe?

Subject Area(s) Life Science

Associated Unit **Cells**

Lesson Title **To Catch a Microbe?**

Header

ADA Description: Green filter Lactobacillus chain
Source/Rights: Christina Rutledge
Caption: Captured Lactobacillus

Grade Level 9 (9-12)

Lesson # 1 of 1

Lesson Dependency **None**

Time Required **180 minutes – Lesson has 4 parts and could be shortened by removing parts that are not needed depending on the curriculum of the course**

Summary
Teaching about the differences between prokaryotic and eukaryotic cells can be challenging because students are, for the most part, unable to associate real things they see with prokaryotic cells. There are four parts to this lesson that focus on allowing the students the opportunity to actually see bacteria and then apply their knowledge to a real life biomedical engineering problem. Students will first stain and view bacteria with traditional stain mechanisms. After they have developed an understanding of what bacteria actually look like with the bright field optical microscope, they will analyze experimental results of pictures of captured bacteria during an experiment at the University of South Florida (USF). In the experiment they will analyze, experimenters are attempting to capture/detect bacteria from low concentration solutions. This has a clinical application that will allow doctors to detect bacteria in transplant samples, such as bone marrow. Currently detection of bacteria in bone marrow takes several days and most facilities do not have the equipment needed to store the bone marrow for this amount of time. This results in patients sometimes receiving a transplant that is contaminated with bacteria and may result in sepsis. As part of their analysis, students will complete a t-test in order to determine the optimal

design for the capturing methods presented. After analysis, students will develop their own experiments to see if they can efficiently capture/detect the bacteria. This part of the lab is inquiry by design, but teachers can put limitations on the experiment by providing a list of available materials. Once students have completed their trials, they will use a phase contrast microscope to analyze their designed capturing methods. After they finish the inquiry portion, they will practice calculating magnification of real prokaryotic cell images. Skills learned in the lesson are calculating magnification of cells, using bright field and phase contrast optical microscopes, describing cell type characteristics, performing statistical analysis of sample sets and using proper bacterial stain technique, creating data tables, graphing data and using scientific method to develop and inquire about scientific principles.

Engineering Connection

Finding an efficient way to detect bacteria in a clinical setting is a great challenge of the biomedical engineering field. People who are awaiting a bone marrow transplant often have a compromised immune system, yet doctors do not have a timely way for bacterial detection of samples before introducing them to the patient. Biomedical engineers can use materials to fabricate filters, or chemical adhesives that may be able to capture bacteria and allow for a quick detection.

Engineering Category =

Choose the category that best describes this lesson's amount/depth of engineering content:

1. Relating science and/or math concept(s) to engineering

Keywords

Bacteria, Microfluidics, Ceramics, Prokaryotic, Eukaryotic, Antibiotics, Biomedical

Educational Standards (List 2-4)

State STEM Standard:

Compare and contrast the general structures of plant and animal cells. Compare and contrast the general structures of prokaryotic and eukaryotic cells.

(Grades 9 - 12)

National Science Education Standard:

Cells have particular structures that underlie their functions. Every cell is surrounded by a membrane that separates it from the outside world. Inside the cell is a concentrated mixture of thousands of different molecules which form a variety of specialized structures that carry out such cell functions as energy production, transport, of molecules, waste disposal, synthesis of new molecules, and the storage of genetic material.

(Grades 9 - 12)

Content Standard A: As a result of activities in grades 9-12, all students should develop Abilities necessary to do scientific inquiry Understandings about scientific inquiry

(Grades 9 - 12)

ITEEA Standard

Standard 13. Students will develop abilities to assess the impact of products and systems.

(Grades K - 12)

NGSS Standard (strongly recommended)

Analyze data using tools, technologies, and/or models (e.g., computational, mathematical) in order to make valid and reliable scientific claims or determine an optimal design solution.

(Grades 9 - 12)

[CCSS Standard] (strongly recommended)

Use statistics appropriate to the shape of the data distribution to compare center (median, mean) and spread (interquartile range, standard deviation) of two or more different data sets.

(Grades 9 - 12)

Pre-Requisite Knowledge

1. How to use a light microscope
2. Explain the importance of standard deviation
3. Lab safety procedures

Learning Objectives

After this lesson, students should be able to:

- **Describe distinguishing structures of prokaryotic and eukaryotic cells**
- **Explain why the structures of different types of cells are important**
- **Calculate magnification of an image**
- **Use statistical analysis to analyze data**
- **Calculate standard deviation and t-test**
- **Properly use an optical microscope, including oil immersion**
- **Analyze data to determine an optimal design solution**

Introduction / Motivation (5E-Engage)

Bone marrow transplants are used to replace nonfunctional bone marrow cells with healthy ones. Usually for a bone marrow transplant, the recipient has a compromised immune system and any introduced pathogens pose a risk. Currently, there is a need for a bacterial detection method that will allow scientists to quickly test bone marrow for microbes before donating to the recipient.

Can we create a glue to selectively capture bacteria? Why are we able to capture bacteria apart from the eukaryotic cells?

Lesson Background & Concepts for Teachers (5E-Explain)

Since the size of a prokaryotic cell is generally less than 5 microns, many students have trouble associating "real" things with the bacteria. This lesson is designed to give students to see the bacteria in two different ways. The first method that involves heat fixing and staining helps students to understand that there are two types of cell walls present in bacteria and that is why there will be some pink and some purple bacteria in their microscope. During heat fixing, be sure students don't allow the slide to get too hot as this will damage the bacteria. You can modify this section if you are only having them stain lactobacillus, which is gram positive. Personal protective equipment should be worn during this activity. Make sure the bacteria you are working with is non-pathogenic. You can culture lactobacillus by boiling milk, allowing it to cool, then adding a small amount of yogurt to the milk and allowing it to set for a day or two. This solution will often have yeast in it as well (which students will see as round structures in the microscopes). If you want to reduce the amount of yeast, take a small diluted amount of the milk yogurt solution and transfer to a petri dish. You can also order non-pathogenic bacteria from your science supply company. Even though you will be working with non-pathogenic bacteria, it will be important that no students are in the lab who have compromised immune systems and that all lab areas and equipment are cleaned with a 10% bleach solution.

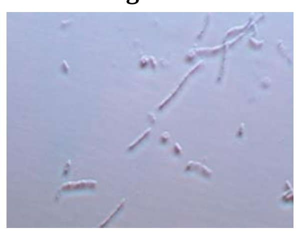
For the inquiry portion where the students set up their own capturing methods, students can use a homemade glue. This can be made using a few basic household chemicals and gelatin. Here is a link for how to make the initial glue: http://www.ehow.com/how_8772806_make-gelatin-glue.html. Students can try different techniques to apply the glue as an independent variable if they would prefer to use the glue the teacher makes. The images that you see were from glue being spin coated on the glass slide with a centrifuge. Most high school labs will not have a vacuum centrifuge that will allow spin coating, but glue can be applied by scraping with a second glass slide, or by tilting the slide and allowing it to run off.

This portion can be set up for students to modify glue made by the teacher, or for students to research their own methods for making glue depending on the level of the course. Some options for changing the glue include pH, gelatin thickness, and adding other compounds such as vancomycin to increase capturing abilities.

Student should be comfortable with the meaning of standard deviation and importance of statistical analysis.

Image 1

Figure 1



ADA Description: Picture of lactobacillus under 1000x magnification

Caption: Figure 1. Lactobacillus on a glass slide using 100X dry lens magnification and phase contrast.

Vocabulary / Definitions

Word	Definition
Prokaryotic	Unicellular organisms that lack membrane bound organelles
Eukaryotic	Organisms which have membrane bound organelles
Magnification	The degree to which something has been enlarged

Associated Activities (5E-Explore)

See Attachments: “To Catch a Microbe”

Lesson Closure

Assessment (5E-Evaluate)

Pre-Lesson Assessment
Descriptive Title: Cell Types Pre-Assessment

Lesson Summary Assessment
Descriptive Title: Completed final lab report

Homework
Descriptive Title: Finish lab calculations and final report

Lesson Extension Activities (5E – Extension)

Students can do their own research on bone marrow transplants, infection and bacterial detection methods. This website has good statistics and information showing just how important this research is.

http://emedicine.medscape.com/article/1013470-overview#a1

Additional Multimedia Support

References

1. Rupesh Chawla, MD. "Infections After Bone Marrow Transplantation" Updated June 27, 2013. http://emedicine.medscape.com/article/1013470-overview#a1

Attachments

"To Catch a Microbe" –student handout

"Cell Types Pre-Assessment"

Other

Redirect URL

Contributors

Christina Rutledge

Supporting Program

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Classroom Testing Information

ATTACHMENTS

To Catch a Microbe

Certain patients with cancers such as leukemia and lymphoma may be recommended by their doctors to have a procedure done called a bone marrow transplant.   Bone marrow is soft tissue inside some bones that contains stem cells. These stem cells have the ability to mature into healthy blood cells. In a person with cancer, or someone who has undergone chemotherapy and radiation, these cells may not be functioning properly. In this case, a bone marrow transplant would attempt to replace the damaged cells with healthy cells.

Patients who are preparing for bone marrow transplants often have compromised immune systems. Unfortunately, doctors do not have a way to test bone marrow for bacteria prior to the patient receiving it. Minor bacterial infections can result in sepsis, a dangerous condition taking the lives of 215,000 Americans each year. Once marrow is taken out of the donor, it is possible to store it for testing, but this technique is expensive and not available in many facilities. This means that in most cases, the bone marrow must be taken from the donor and transferred to the recipient in a short amount of time. This poses a serious risk to patients receiving the bone marrow transplant, since they already have a compromised immune system. Scientists at the University of South Florida (USF) are testing two methods to capture and detect bacteria from fluids in only a matter of hours. You will be examining some results of the capturing abilities for each method and determining which method is best and how to improve capturing of bacteria. You will also be doing your own research and developing an experiment to see if you can capture bacteria.

Part 1: Determining the Structures of Bacteria

First, you will stain and view bacteria with a microscope to gain a better understanding of the structures you will be looking for. Since prokaryotic cells are only about 5 microns, we will need to use a microscope that can magnify samples 1000x and the lens we use for this requires oil immersion. Once you have made and stained your prokaryotic slides, you can focus on a lower power lens. Once in focus, you add a drop of oil to the top of the slide and switch to the highest power. Please note that oil should not be used with any lens other than the 100X oil. Even at this magnification, we are only able to see shapes of the bacteria and very few actual structures.

Gram Stain Procedure—

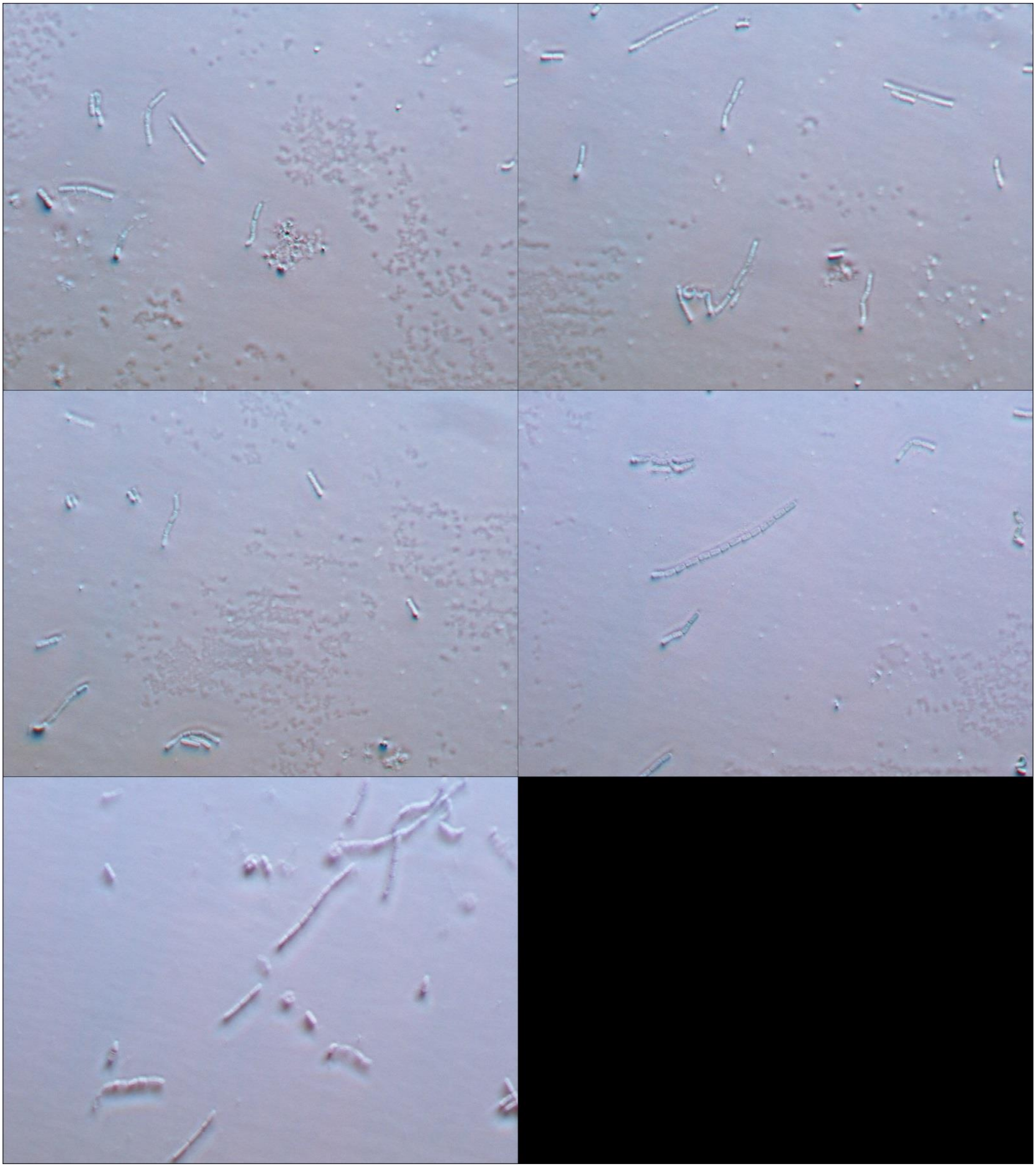
Safety precautions:

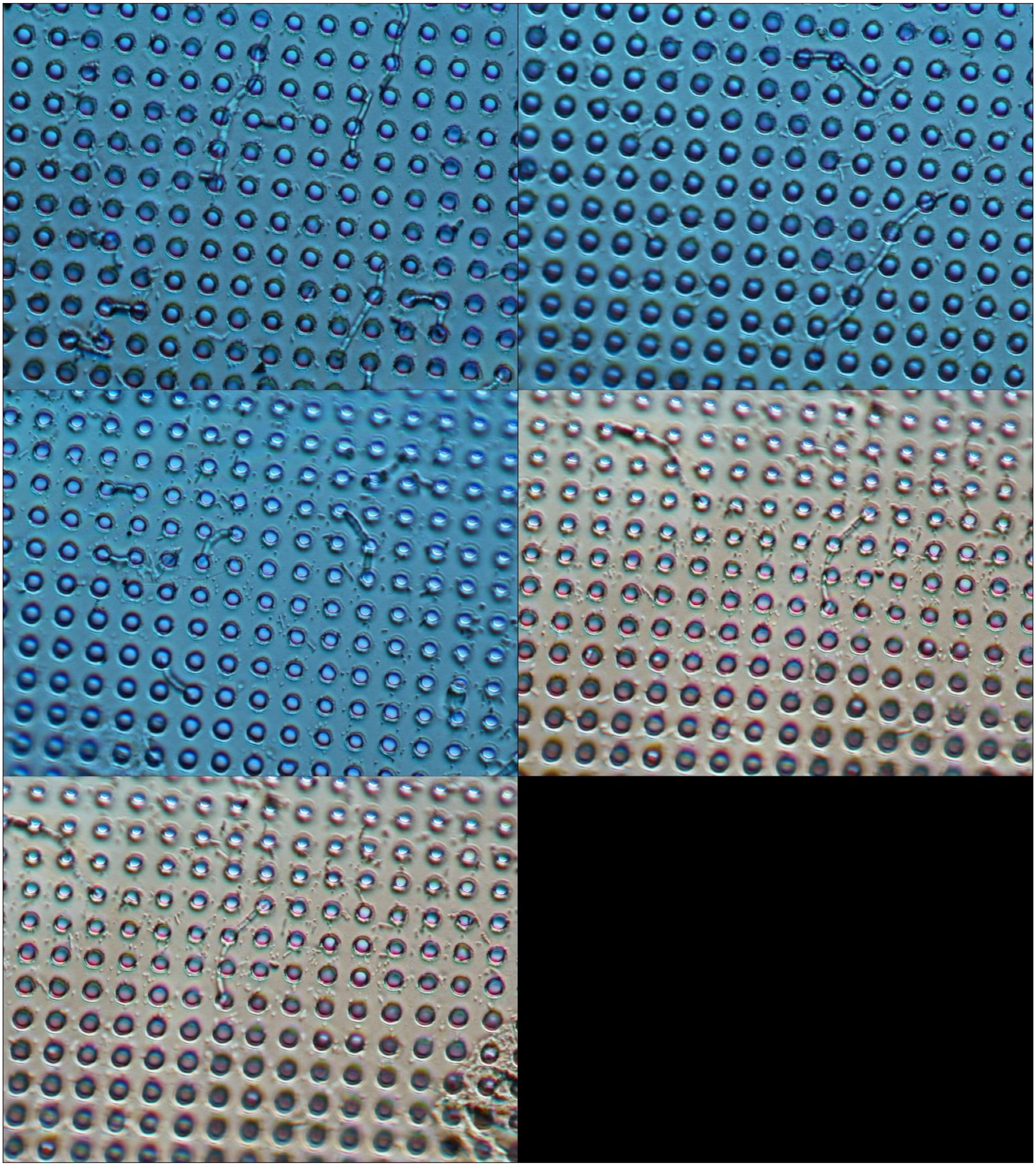
- ✓ *While the bacteria you are working with is considered non-pathogenic, it is very important to follow proper procedures for handling biological hazards. This lab also involves an open flame and proper safety precautions for working around a flame should also be followed.*
- ✓ *Proper protective equipment such as aprons, gloves, goggles and close toed should be worn.*
- ✓ *All surfaces and materials should be cleaned with a 10% bleach solution at the end of the lab.*
- ✓ *Hands must be washed before leaving the lab.*
- ✓ *Be sure not to touch your face, clothing, cell phone etc. with contaminated gloves.*
- ✓ *All long hair should be tied back*

1. Use a sterile inoculating loop to collect a small amount of bacteria and transfer it to the microscope slide. It should be a *very* thin film. Clumped bacteria will not be as visible in the microscope.
2. Hold the microscope slide with tongs. Dry and fix the smear by passing it through an open flame for one second. Wait 10 seconds and pass again if it is not completely dry. If you hold the smear in the flame you will burn the bacteria and will not see any on your slide!
3. Add a few drops of crystal violet stain. Let stand for about a minute.
4. Pour off excess stain and dip slide into water (or hold carefully under VERY slow running water) to carefully rinse stain from slide.
5. Drain excess water, cover smear with iodine solution. Let stand for 1 minute. Pour off iodine and rise as before.
6. Holding slide at a 45 degree angle, place 95% denatured ethyl alcohol on the slide and allow the alcohol to flow across the smear. Add dropwise until the drops are clear.
7. Drain excess water and cover the smear with Safranin O solution. Let stand 1 minute.
8. Rinse again and blot dry.
9. View with a high power microscope and draw bacterial structures. (use oil immersion on the highest power only)

Part II: Determining the Best Capturing Method

Once you have become familiar with the structures, you will need to examine the images captured from the USF lab and count the captured bacteria from each method. To get more reliable results, the researchers have sent you 5 fields of view for each capturing method. This will allow you to count the bacteria, find average number of captured bacteria for each method and then perform a t-test. From the t-test, you will suggest whether or not there is a significant difference between the capturing methods and if there is a difference, suggest which method is the optimal design.





Performing a t-test Procedure—

1. Calculate the Standard Deviation for each set of data. Here is the formula, to use it, plug the numbers into the tables below.

$$s = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Data Set 1			
X (number of bacteria)	$\bar{x}$ (avg)	$x - \bar{x}$	$(x - \bar{x})^2$
		$\sum (x - \bar{x})^2$	

Data set 1

$$\frac{\sum (x - \bar{x})^2}{n-1} = \_\_\_\_\_\_\_\_\_\_\_\_ = s$$ (Standard Deviation)

Data Set 2			
X (number of bacteria)	$\bar{x}$ (avg)	$x - \bar{x}$	$(x - \bar{x})^2$
		$\sum (x - \bar{x})^2$	

Data set 2

$$\frac{\sum (x - \bar{x})^2}{n-1} = \_\_\_\_\_\_\_\_\_\_\_\_\_\_ = s \text{ (Standard Deviation)}$$

2. Calculate the variance (var) for each sample, which is equal to s^2

   Data set 1 var ($s^{2)}$ = ____________

   Data set 2 var (s^2) = ____________

3. Calculate the Standard Error of Mean (to test and see if the mean of the 1st sample is significantly different than the mean of the 2nd sample)

$$SEM = \sqrt{\frac{\text{var}_1}{n_1} + \frac{\text{var}_2}{n_2}}$$

   SEM= ___________

4. Now you're ready to calculate the t-statistic

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SEM}$$

   t = ____________

5. Now that you have your t-value, you can use the chart to determine if your two sets of data are significantly different (statistically significant). The probability (p) that is generally used by the scientific community is 0.05. A p value of 0.05 means that there is a 95% probability that the hypothesis is correct.

   To use the table, find the degrees of freedom for your data set. Since we are comparing two means, the number of degrees of freedom is $(n_1+n_2)-2$.

Degrees of Freedom	Probability, *p*			
	0.1	**0.05**	**0.01**	**0.001**
1	6.31	12.71	63.66	636.62
2	2.92	4.30	9.93	31.60
3	2.35	3.18	5.84	12.92
4	2.13	2.78	4.60	8.61
5	2.02	2.57	4.03	6.87
6	1.94	2.45	3.71	5.96
7	1.89	2.37	3.50	5.41
8	1.86	2.31	3.36	5.04
9	1.83	2.26	3.25	4.78
10	1.81	2.23	3.17	4.59

Part III: Developing Your Own Capturing Method
**All experiments must be pre-approved by the instructor*

Now you develop an experiment to see if you can capture bacteria. One of the methods used at USF included a homemade glue from mainly gelatin and water. You should first do some brainstorming about the structures of a bacteria and how you might exploit these structures to capture the bacteria. You can use a micropipette to pass a small amount of diluted bacterial solution over the top of a glass slide covered with your glue, or capturing method. (use of a glass slide and glue are not required) You also have the option to change the composition of the glue to get different properties you are interested in. In the USF lab, they added antibiotics with bacterial binding properties to enhance capturing. You should consider surface chemistry and remember that your capturing method will only be useful if you can still identify the presence of bacteria with a microscope. You have two options available for the posterior microscopy. If you are able to stain your samples at the end of your test, then you can use the bright field microscope with the 100X oil immersion objective. If your sample cannot be stained at the end of your test, you will use the phase contrast microscope. Both are optical microscopes, but the phase contrast has achromatic objectives and a special condenser below the stage that optically maximize the diffraction of light from your bacteria. Remember that the goal of this research is to find and detect bacteria in a short amount of time, so culturing your samples will not be an option.

Scientific Method Check-List

A hypothesis is stated in correctly stated.

( ) yes ( ) No, because

____

There is a control group.

( ) Yes ( ) No, because

____

The independent variable is correctly identified.

( ) Yes ( ) No, because

____

The dependent variable is correctly identified.

( ) Yes ( ) No, because

____

The control group differs from the experimental group by only one variable and the other variables are the same in all groups.

( ) Yes ( ) No, because

____

The procedure is detailed clearly stated.

( ) Yes ( ) No, because

____

The experiment is feasible and safe.

( ) Yes ( ) No, because

____

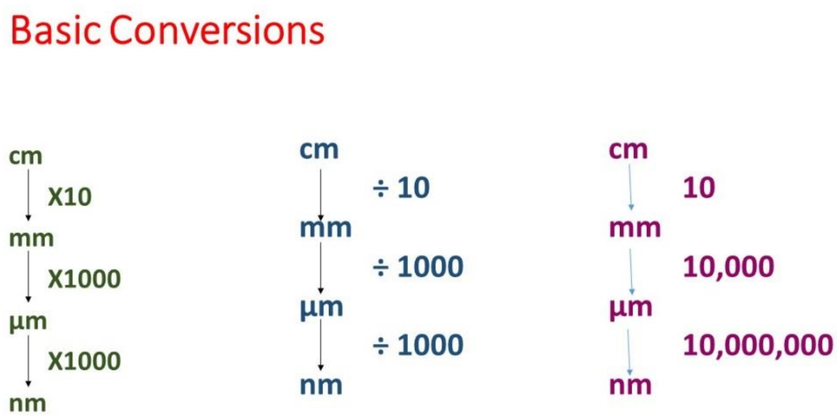
Teacher Signature (Must have before beginning your experiment!)

____

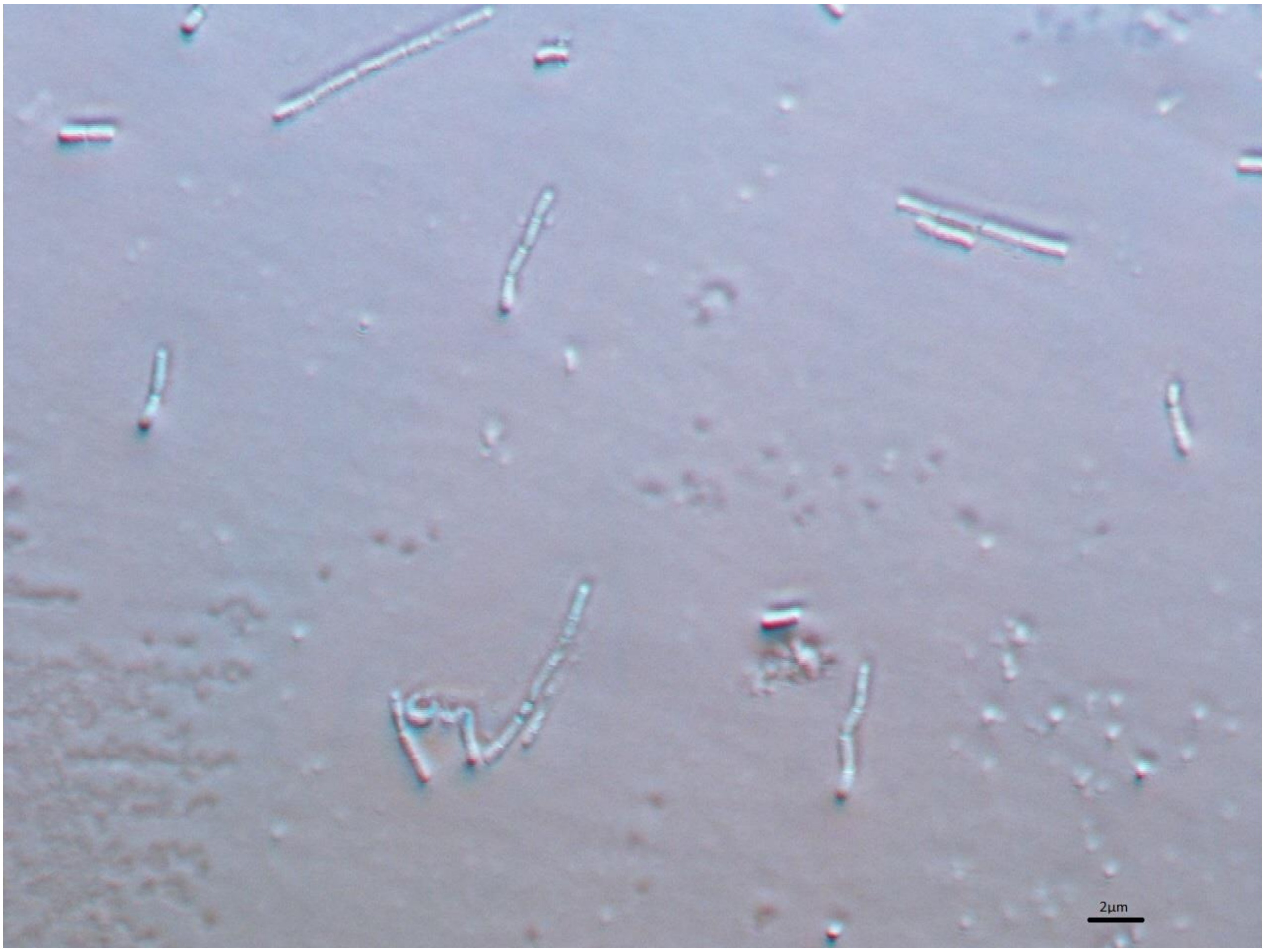
Create a report that explains in detail the design of your experiment (procedure), shows your variables and how they were controlled. Include a raw data table, processed data table (where applicable) and present processed data appropriately. Be sure to use either a t-test, or standard deviation with error bars, to process your data. If you are comparing two situations, then it would be most appropriate to use a t-test to determine if there is a significant difference between the two sets of data. If you set your experiment up as a 5x5 model, it would be most appropriate to use standard deviation and error bars.

Part IV: Calculating Magnification

You will then perform image analysis by measuring the image size and calculating magnification of bacteria in the images. Remember the units of your measurement and the scale bar must be the same to perform the calculation properly.



Circle the bacterium you plan to measure, then calculate the image magnification for that bacterium. Show your work below.


2µm

ATTACHMENT 2
CELLS TYPES PRE-ASSESSMENT

Cell Types Pre-Assessment

Multiple Choice
Identify the choice that best completes the statement or answers the question.

____ 1. One difference between prokaryotes and eukaryotes is that
- a. nucleic acids are found only in prokaryotes.
- b. mitochondria are found in larger quantities in eukaryotes.
- c. the Golgi apparatus is found only in prokaryotes.
- d. prokaryotes have no nuclear membrane.

____ 2. Which of the following is characteristic of prokaryotes?
- a. They have a nucleus.
- b. They existed on Earth before eukaryotes.
- c. The organelles in their cytoplasm are surrounded by membranes.
- d. None of the above

____ 3. Which of the following is an example of a prokaryotic cell?
- a. an amoeba
- b. a virus
- c. a bacterium
- d. a liver cell

____ 4. Only eukaryotic cells have
- a. DNA.
- b. membrane-bound organelles.
- c. ribosomes.
- d. cytoplasm.