Part 1: Aseptic Technique & The Streak-Plate Method of Isolation Follow-up

Theory:

Retrieve your two Petri dish plates from the last lab and evaluate if there were any problems or successes with your ability to obtain isolated colonies after the Streak-Plate Method. Notice any problems that occurred. You can answer question 1 while you wait for lab to begin. Save your plates for Part 2 of today's lab where you will observe what you grew under the microscope using a simple staining method.

Part 2: The Microscope & The Simple Staining Technique

Theory:

Millions of microscopic structures and organisms can be easily seen with the light microscope. The first few tasks will be to familiarize yourself with its basic function, and then to view some actual microorganisms. **When in doubt about how to fix a focusing problem, always return to the lowest total magnification, get things in focus there and then proceed to the higher magnifications. Also, remember that more light is needed at the higher powers to improve the resolution**. All used slides and coverslips are thrown away in the white waste receptacle.

Part 2 Procedure:

Lab Materials:

Amount per Student	Material
1	Microscope (Stored in cabinet in the lab. Okay to share with 1 lab partner. Return it when done.)
1	**MICROMETER SLIDE (return to tray when done — DO NOT ACCIDENTALLY THROW IT AWAY !!)**
1	**NEWSPRINT SLIDE (return to tray when done — DO NOT ACCIDENTALLY THROW IT AWAY !!)**
as needed	Blank Slides and Coverslips (at your station, or nearby. Discard in the glass waste receptacle when done.)
as needed	Immersion Oil
1 or 2	Petri Dishes with streak–isolation of bacteria on it from the previous lab (Discard in red trash when done).
1 or 2 drops	water from Hay Infusion
when needed	Dye (3 or 4 choices are by the sinks)
when needed	Bilbous Paper (located near the sinks and used for drying slides)
1	Inoculating loop (at your station)
1	bunsen burner (at your station)

Lab 6 Questions (Due at the end of lab)

Name: _____ Grade: _____ of 10 points

1. (1 point) List 2 organisms from last week's lab on normal flora that you likely have on one of your plates and which plate it came from (i.e. *E. coli* from the EMB plate, or *S. aureus* from the MSA plate). Then indicate which one you are using for question 7.

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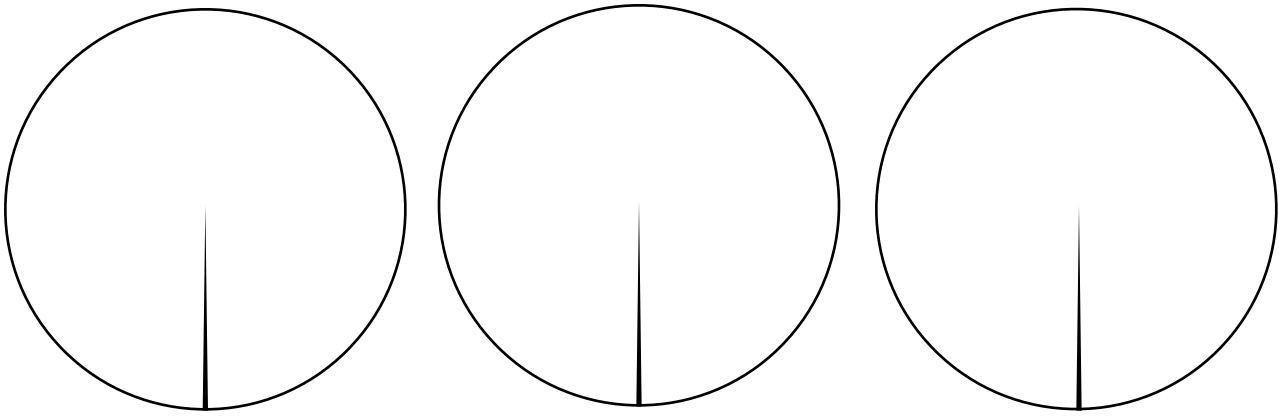
2. (1 point) Calculate the Total Magnification for each objective of your microscope and fill in the chart.

Ocular Lens Magnification	Objective Band Color	Objective / Total Magnification "Nickname"	Objective Lens Magnification	Total Magnification
10X	Red	"Scanning Power"	4X	(10X) (4X) = 40X
10X	Yellow	"Low Power"		
10X	Blue	"High Power"		
10X	White	"Oil Immersion"		

3. (1 point) Using the MICROMETER SLIDE, estimate the DIAMETER (in µm) of each field of view. Show any work, then fill in the chart with your final answer. Remember that 1mm = 1,000 µm.

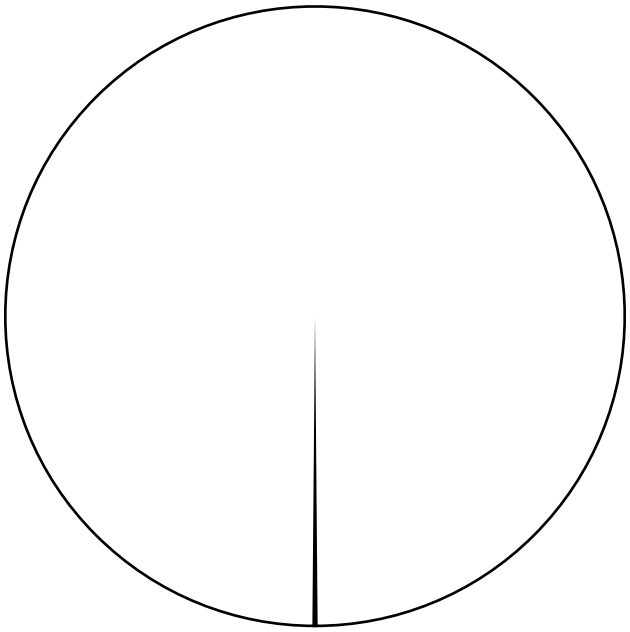
Objective Band Color	Estimated Diameter of Field of View (in µm).
"Red"	
"Yellow"	
"Blue"	
"White"	

4. (1 point) View, then draw a simple sketch any letter on the NEWSPRINT SLIDE at "scanning", "low", and "high" total magnification:



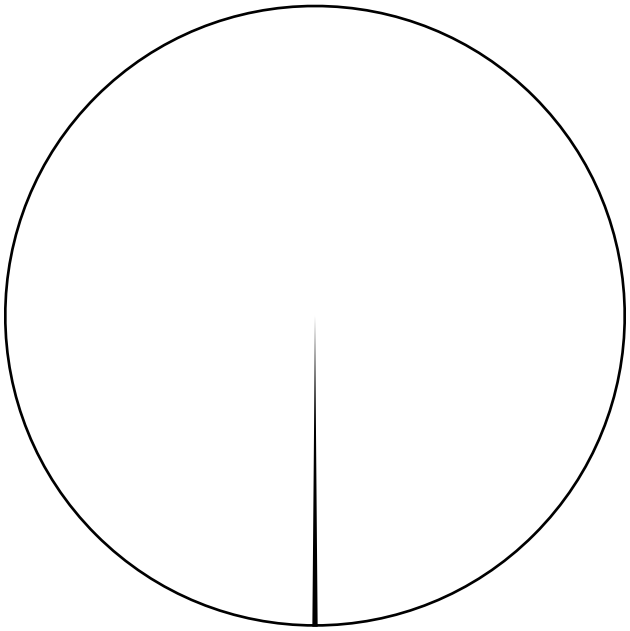
"Scanning Power" "Low Power" "High Power"

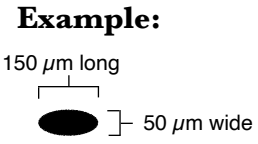
5. (2 points) Use the same sketch of your chosen letter from question 4 at "Low Power" and reproduce it below. Use the estimated diameter of the Field of View from question 3 for the "yellow" objective lens and calculate an estimate for the WIDTH of the letter in your sketch in μm. Show your work / math.



Width of Letter in μm at "Low Power"

6. (2 points) On a clean slide, place 1 or 2 drops of the water from the Hay Infusion and place a single coverslip on top. Sketch any organisms you see and provide a length or diameter measurement (in μm) for one of them. Be sure to write in the total magnification you are sketching the organism at. If any are moving too fast to view, you can use a drop of "Protoslow" to slow them down to better make your sketch and measurement estimates. Show your work.

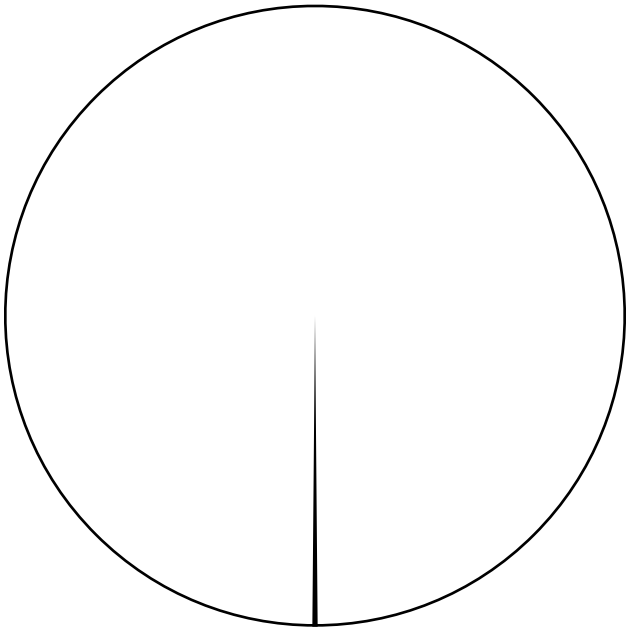




Length and Width of organism in μm at total magnification of your choosing.

7. (2 points) <u>Heat Fixation & Simple Stain Procedure</u>:

1. On another clean slide, use a sterile inoculation loop and take a very tiny sample of one of your bacterial colonies from one of last lab's Petri dishes. You may need to add a single drop of distilled water if the sample is "clumpy" — if adding a drop of water or saline, mix the drop very well into the bacterial clump and spread it very thin.
2. Holding the slide with a clothespin, gently Heat-Fix the bacteria to the slide by passing the slide over the flame till nearly all evidence of moisture is gone. Any area that is not dry will get washed away in the next step!
3. Go to the sink and place a single drop of any dye (such as Methylene Blue). After 30 seconds, rinse any excess dye with distilled water into the sink and blot dry with the bilbous paper pad. It is okay if a little moisture is still present on the slide as it will help the coverslip to stick better.
4. At your lab station, place a cover slip on the slide and view it at "scanning power". Once in focus, progress up each objective lens till you are ready to progress to oil immersion. Just before you move the oil immersion objective lens into place, place a drop of oil on the coverslip and move the objective lens into place. ONLY use the fine focus at this point and make tiny adjustments to get your specimen in focus.
5. *Sketch just some of the bacteria and provide an estimate of the diameter of a single bacteria (in µm) under oil immersion. Be sure to show your work.*



Diameter of bacteria in µm at "Oil Immersion"