A microscopic image of a bacterium, likely a rod-shaped species, showing its internal structure and cell wall. The bacterium is elongated and has a distinct, slightly tapered shape. The background is a light, textured surface, possibly a slide or a culture medium.

Microscopy - Chapter 1

Lecture 3

Bacterial Morphology (Part 1)

Identification Is Based on Morphology

- Organisms were first identified based on what they look like (morphology).
- Disease organisms only need to be identified to species, or subspecies if toxic chemicals or specific antibiotics are to be used to try to kill them.
- Using Dr. Elaine'sTM Soil Food Web Approach techniques, we just need to know what types of bad organisms are present and thus replenish the beneficial organisms that will deal with the bad. Most of the time, morphology is more than adequate to tell us what needs doing.

General Bacterial Classification

“Beneficial” Bacteria

- Suppress Disease
- Build Soil Structure:
Microaggregates
- Retain Nutrients: Water
- Decompose Toxins
- Maintain alkaline soil pH

“Bad” Bacteria

- Cause disease
- Release toxins
- Lower pH (acidic)

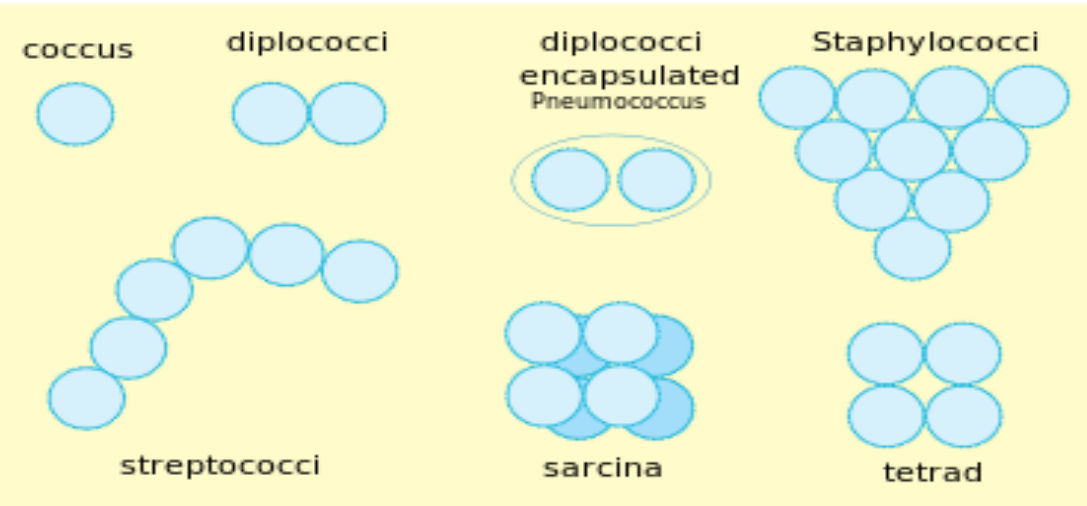


Bacterial Shapes

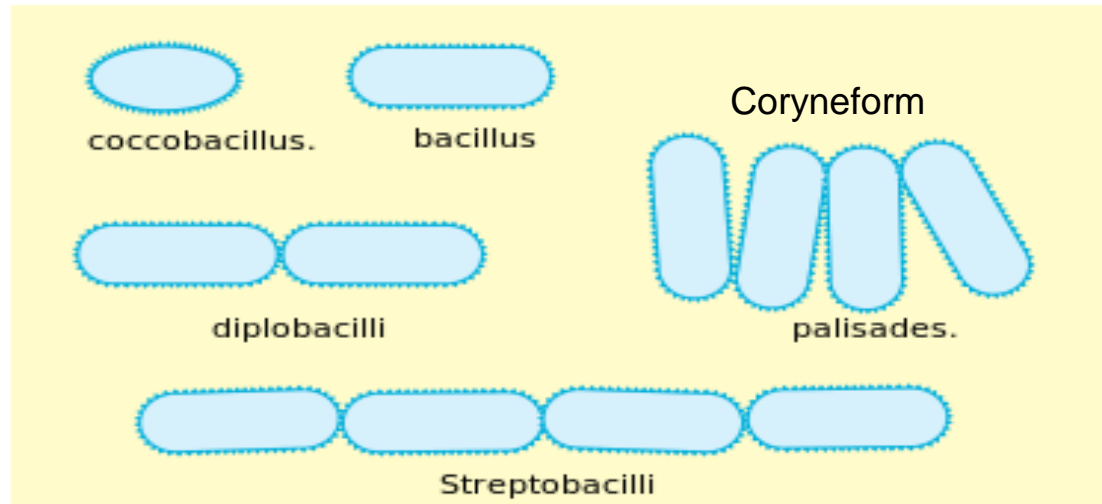
- Bacilli (rod): long, short, fat, thin
- Cocci (round): tiny, small, medium, large
- Coccobacilli (round rods): Single; Double (two in chain called diplo-); in chains (strepto-); Motile or not; Palisade or picket fence (Corynebacteria); Fat, very square rods are the best Bacillus species; Long slender rods (Lactobacillus)
- **Vibrio (comma shaped)**
- **Spirilla (cork-screw shape, stiff, motile)**
- **Spirochetes (move like snakes; flexible)**

Different Shapes of Bacteria

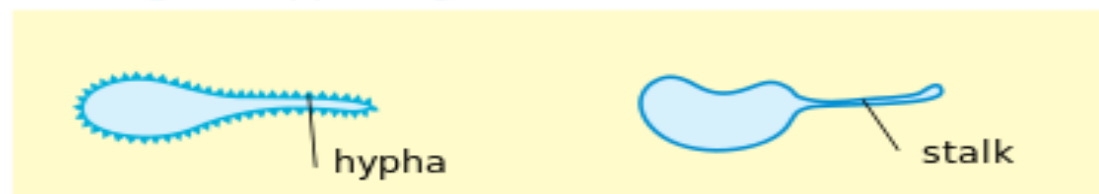
Cocci



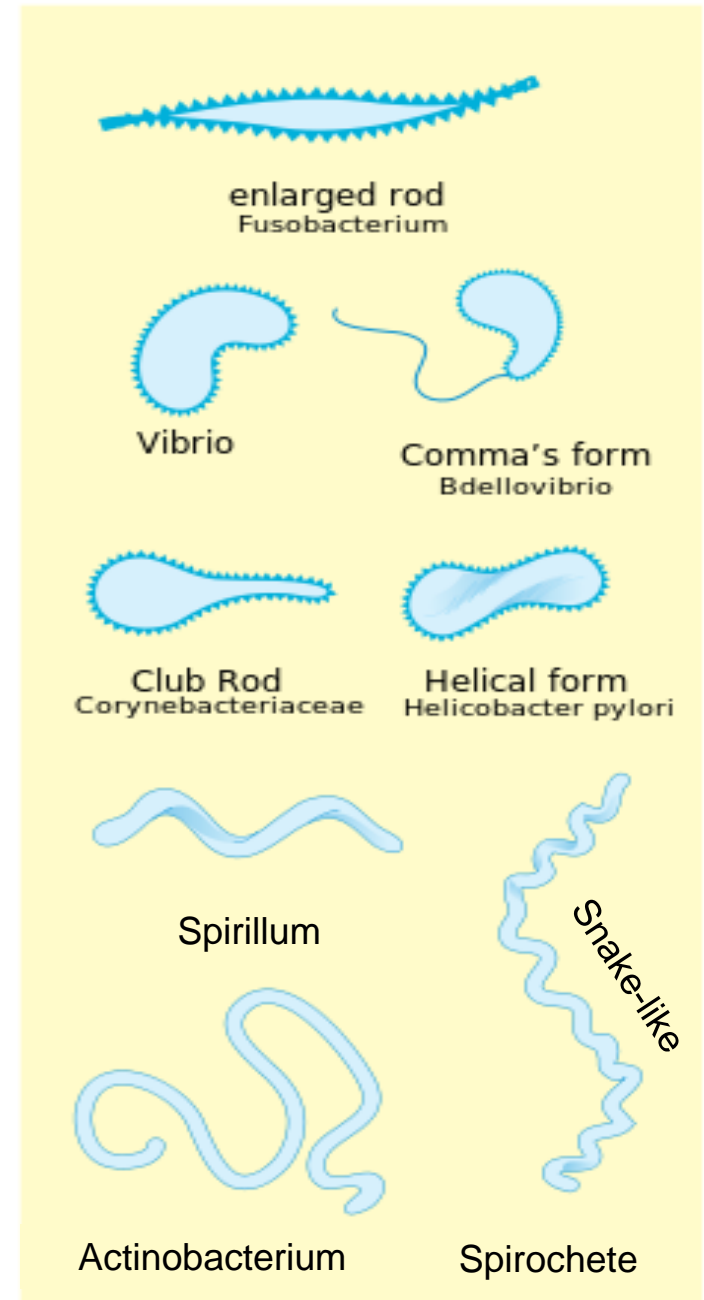
Bacilli

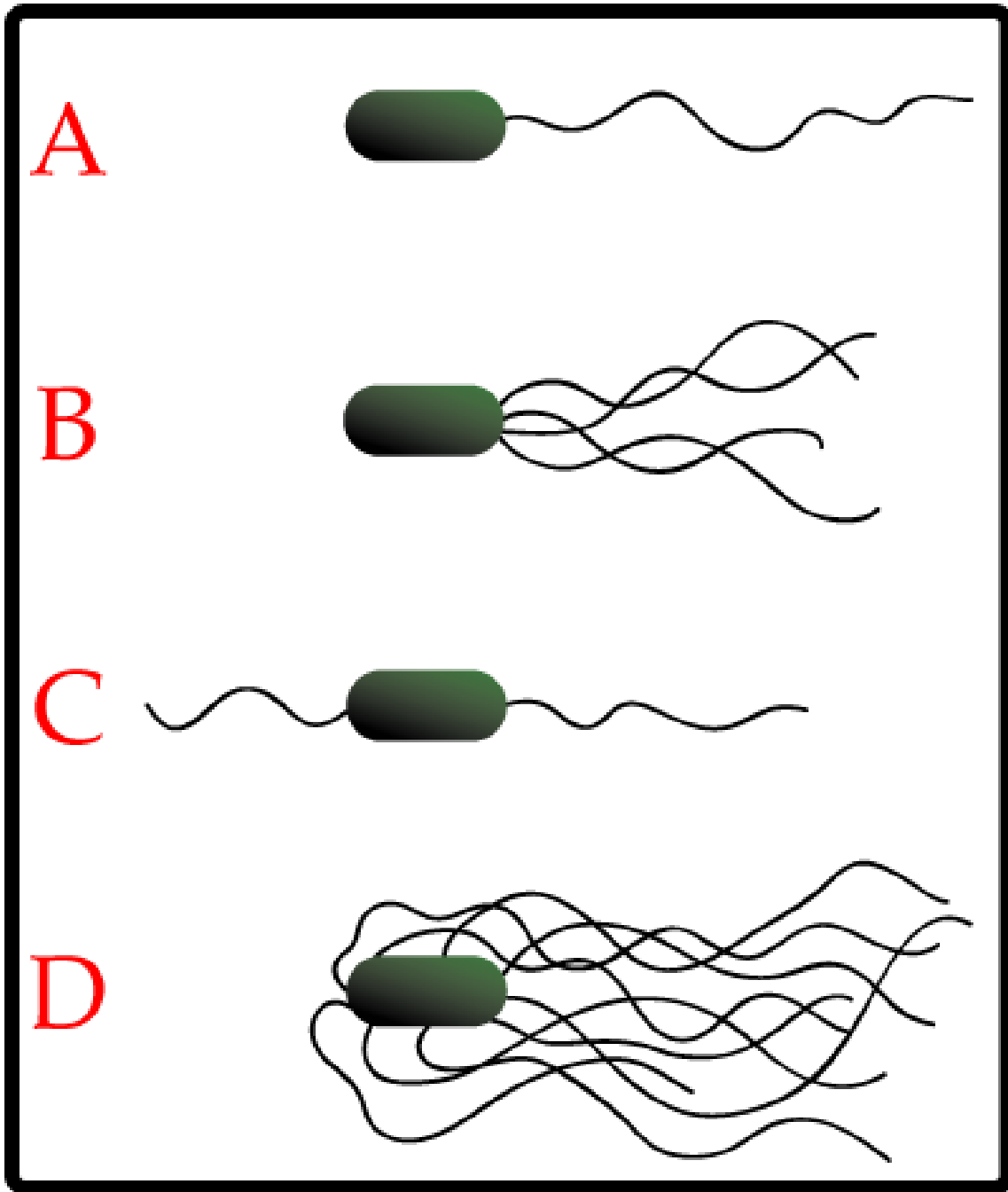


Budding and appendaged bacteria



Others





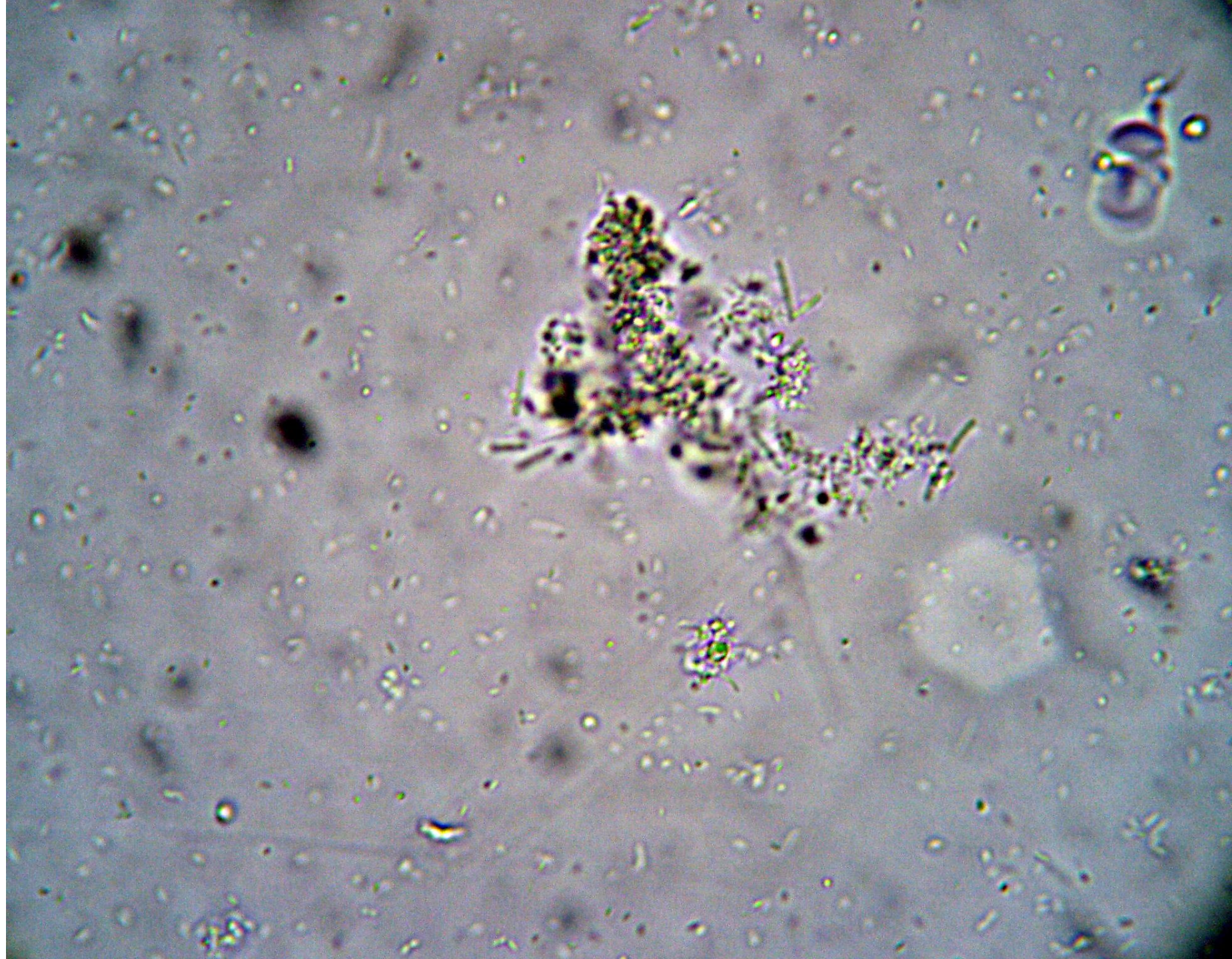
Bacteria can have flagella too! Not all organisms with flagella are flagellates!

But, it takes an electron microscope to see bacterial flagella.

How many bacteria
are in this field?

Diversity?

Upper right corner?



Compare a single tree to a single bacterium

Bacteria are small in size, but extremely numerous.

A single bacterium seems insignificant compared to a single tree, or a single honey bee, or a single microarthropod.

But, taken collectively, bacterial BIOMASS rivals the biomass of all the other organisms in any ecosystem.

We need to compare BIOMASS, not numbers of individuals. So, how do we measure BIOMASS?



Count all those bacteria!?! No way!

How can we count so many bacteria? The task seems overwhelming!

Here's the secret: **Dilution!**

Dilute the sample until the number of bacteria are easy to count!

We make a 1:5 dilution initially, and one drop of that goes on the microscope slide. Observe that drop and decide if there are only 30 bacteria per field, or per quarter field.

Area of the Field of View (FoV)

As discussed a few slides ago, the area of a FoV decreases as magnification increases.

Thus for each objective – eyepiece combination, a FoV will be a different size area.

The only magnification we need to determine, however, is for the 40X objective, the objective used for all the measurements of size.

Here are the calculations to determine FoV area.

Conversion of FoV Area to Coverslip Area

Now we need to know how many FoV are in the area of the Coverslip. Remember that the same units need to be used throughout.

Using an 18 mm by 18 mm coverslip, it's area 18,000 μm by 18,000 μm = 324,000,000 μm^2 .

Divide this by the area of 1 FoV in μm to determine how many FoV are in the one drop under the coverslip.

$324,000,000 \mu\text{m}^2 / \text{FoV } 40\text{X Objective} = \text{Conversion factor.}$

Bacterial Biomass Determination

- A “countable” number of bacteria is around 30 to 50 in a field or quarter of the field.
- Density of bacteria in the initial dilution of 1:5 is usually too high to easily count.
- So, dilute more! Add 5 more ml to the 1:5 dilution. Shake. Has the cloudiness you see in the 1:5 dilution been significantly reduced? If yes, take a drop from the 1:10 dilution, put on the microscope slide, coverslip it and look at how dense the bacteria are. Is this countable?
- If no, then take 1 ml from the 1:10 dilution and place in a clean test tube (continue on next slide).

Bacterial Biomass Determination

- Add 9 ml to the drop of 1:10 dilution in that test tube. Shake a couple times to make uniform.
- This is now a 1:100 dilution.
- Check the 1:100 dilution. Is the dilution nearly clear? If yes, check using the microscope.
- If no, then take 1 ml from the 1:100 dilution, and place in a clean test tube. Since you are getting close to the right dilution, we hope, just add 4 ml more of water to the 1 ml from the 1:100 dilution, to make a 1:500 dilution.
- Check using the microscope. If still too dense, repeat dilution, check if now dilute enough to count.