Microscopy - Chapter 1

Lecture 4

Bacterial Morphology (Part 2)

Bacterial biomass: A Qualitative Approach

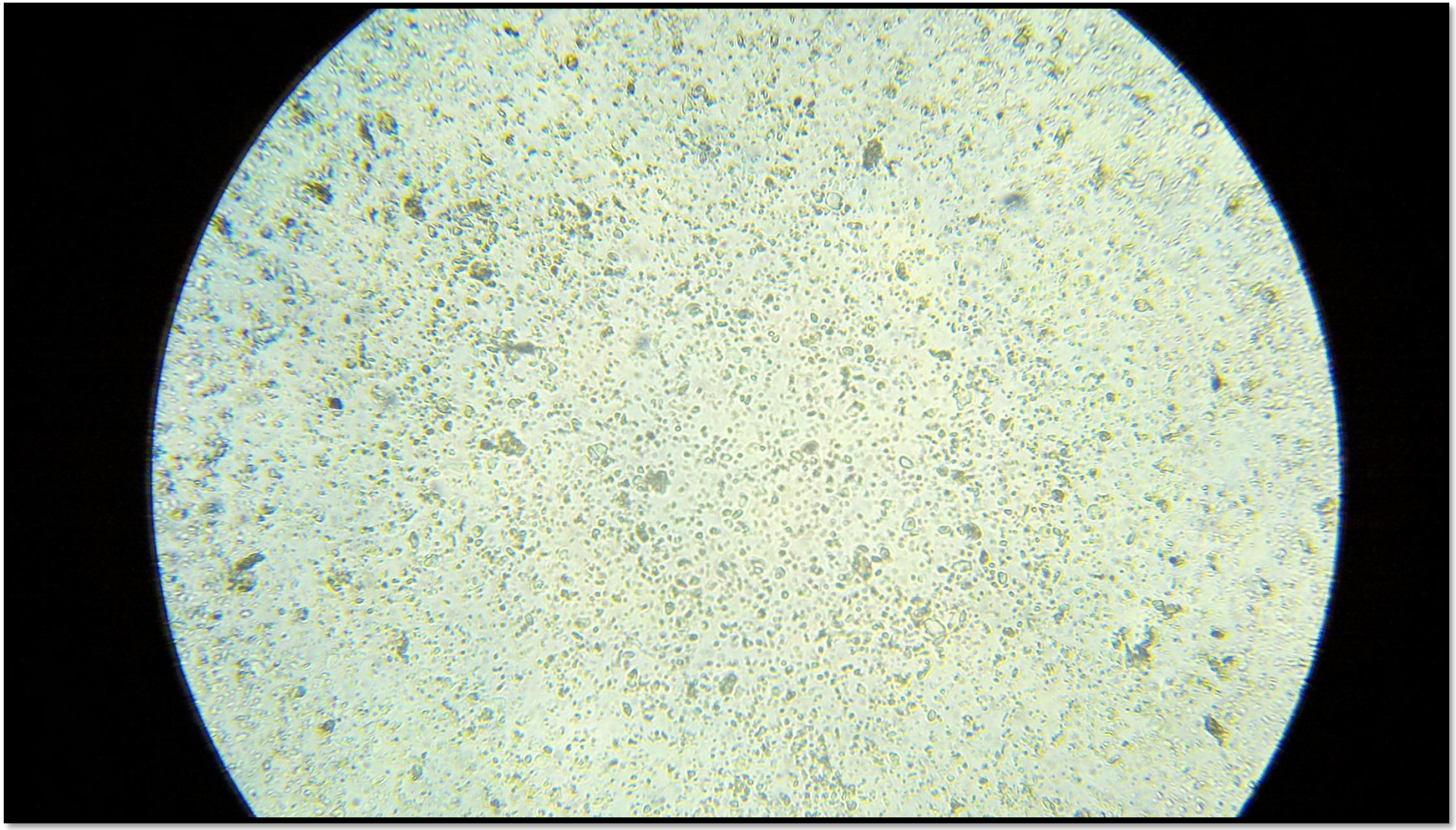
Can we make the estimation of bacterial biomass simpler?

Keisha Wheeler has put together a series of pictures from low bacterial density to high bacterial density.

Can we use a picture of the 1:5 dilution to suggest what the density will be if the complete dilution series is used?

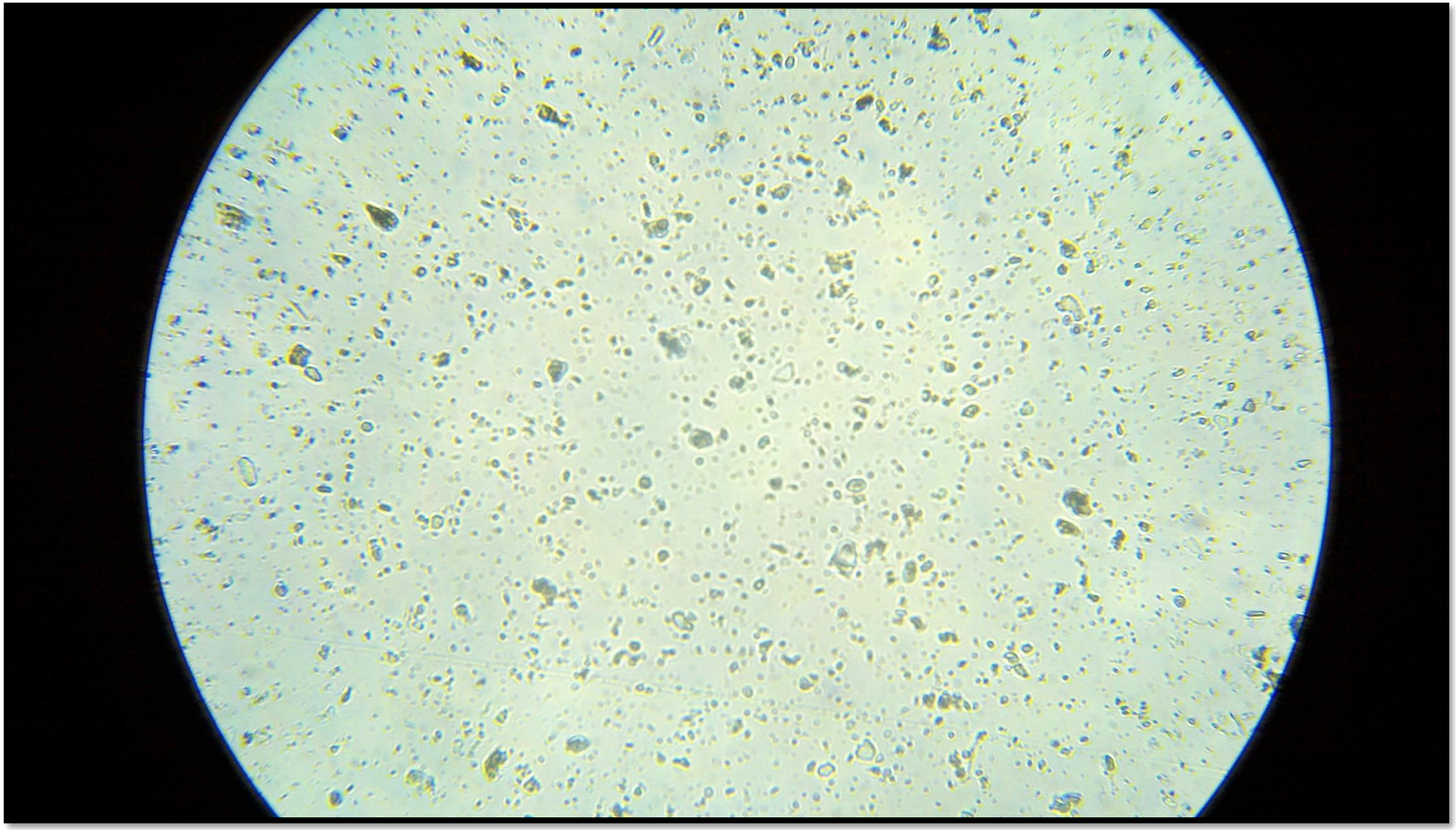
So, here’s the first step to work this out.

1:5 Dilution



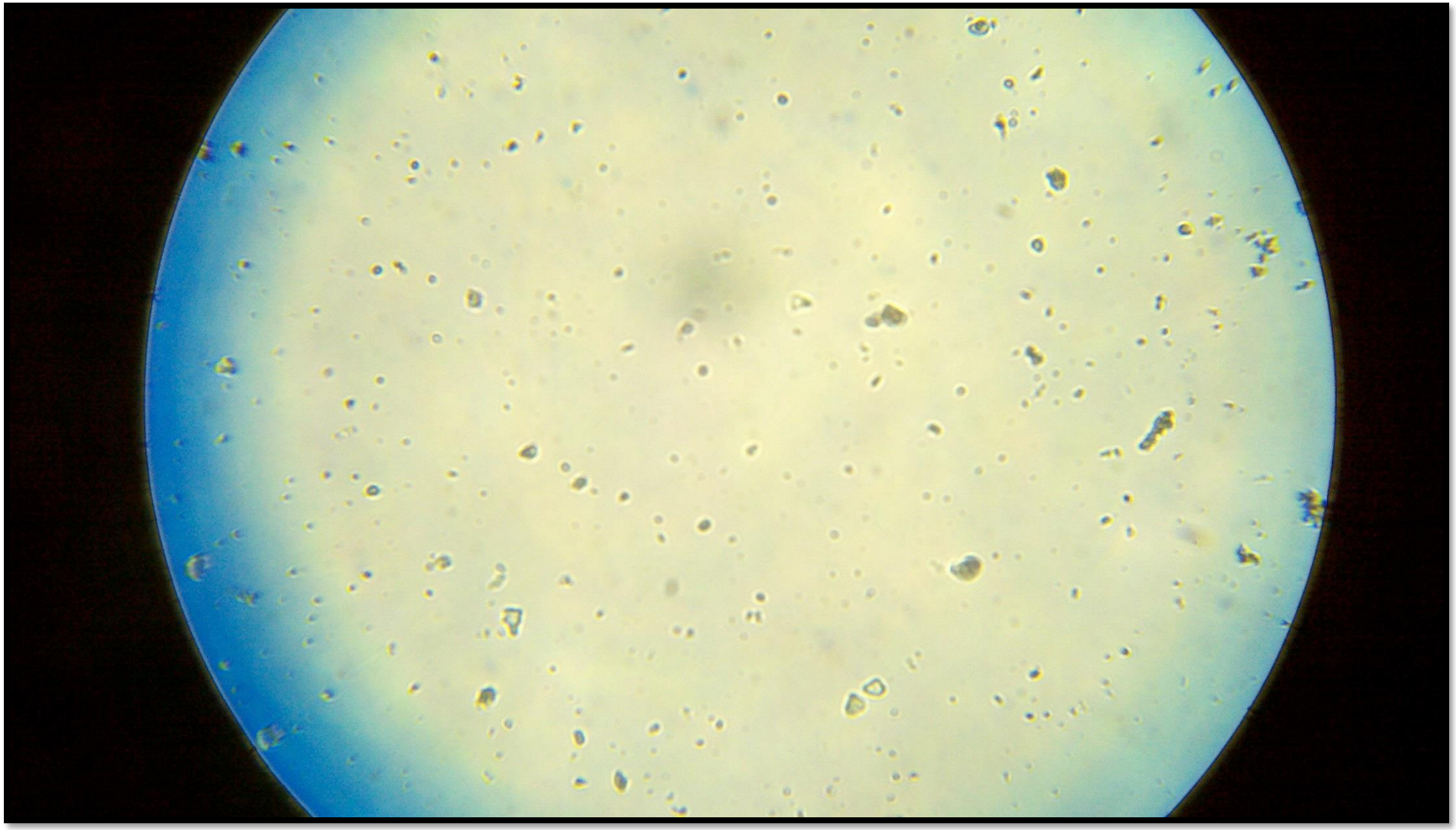
Way too many to count!

1:10 Dilution



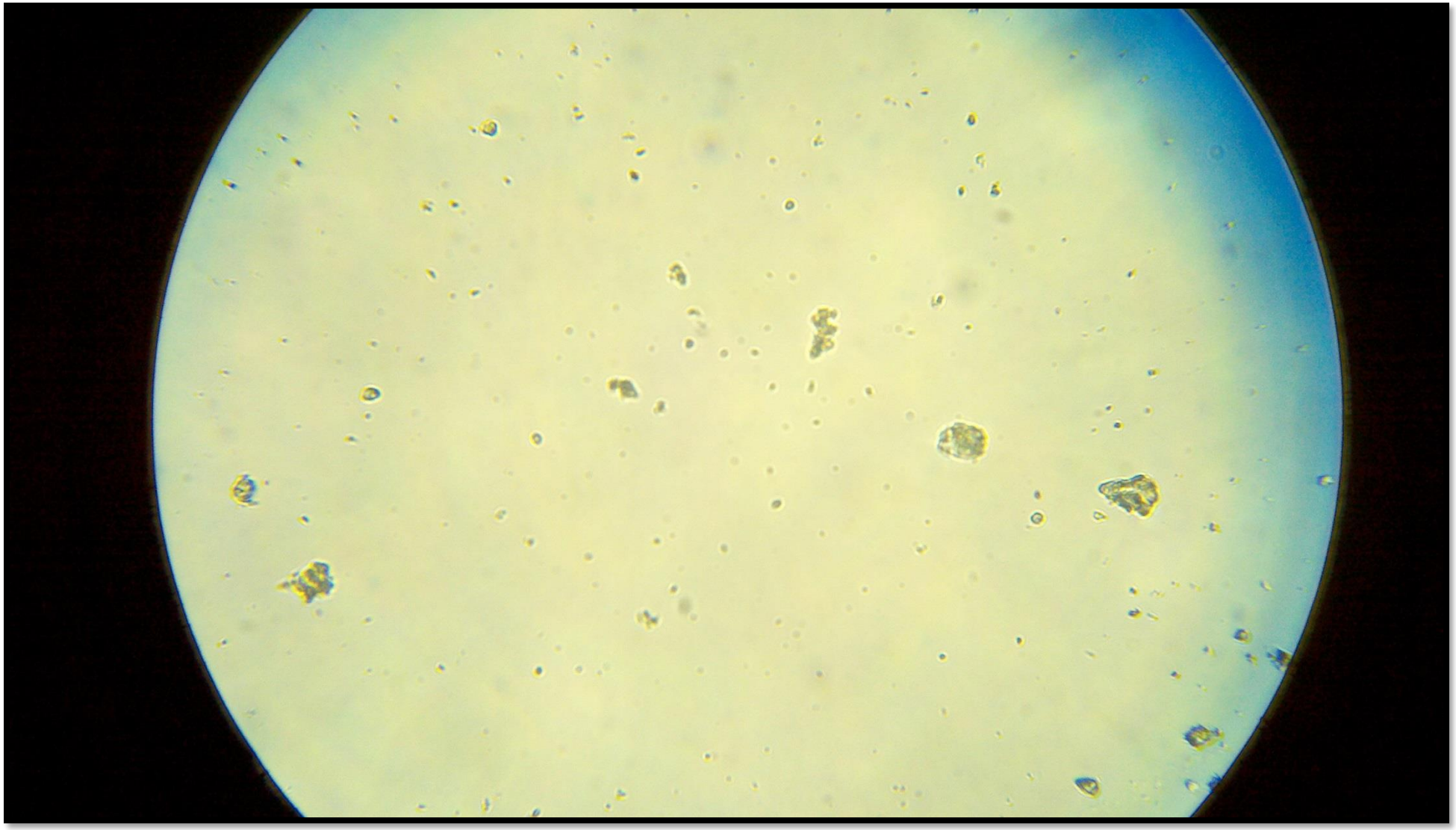
Not countable! Too many!

1:100 Dilution: Bacteria spread out enough?



Avoid counting lumps: focus top to bottom

1:500 Dilution: Count quarter of the field



38 bacteria in ¼ field = 152 bacteria/field

Things to Consider

- Ragged, pointy, not-smooth surfaces are clays, sand grains, silts or organic matter, and are not bacteria. Focus on bacterial density, not on junk!
- FOCUS THROUGH THE COMPLETE DEPTH OF THE SAMPLE!!!! You MUST count all the bacteria, from top to the bottom of the volume between the coverslip and the microscope slide.
- Make your own table. Since mineral particles and organic matter can be much higher or lower in your soil, make your own sequence of pictures and determine the actual relationship between bacterial biomass and dilution. We may need a different table for your soil versus compost versus extracts and teas.

Observe density at each dilution

Now let’s look at the dilution series Keisha assessed.

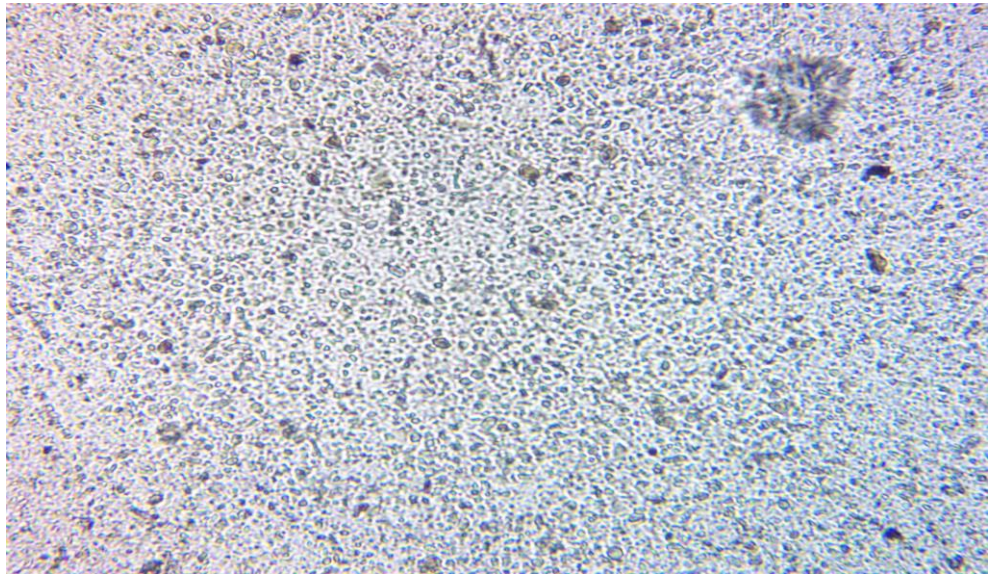
If we consolidate Keisha’s data with your data, or if you develop a similar table for your own use, you would only want to use the estimates for growers who want reasonable accuracy, but rapid turn-around is more important.

This method will likely be modified as our experience increases. Stay tuned to the Soil Food Web community for updates!

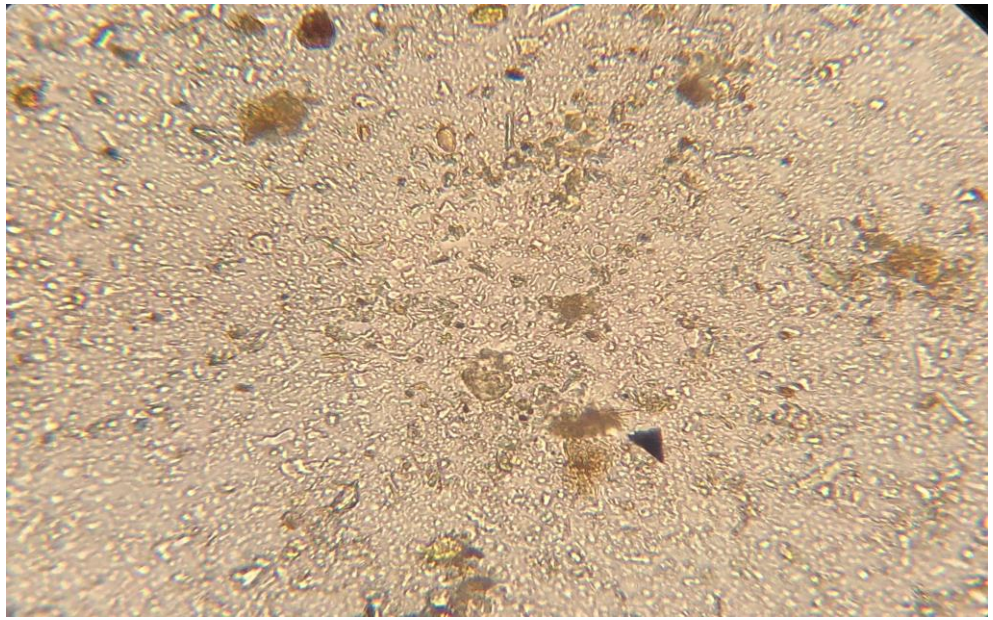
The Conversion Table:

- On the left side is the picture taken at 1:5 dilution
- On the right side is the value determined at the correct dilution, based on 5 fields of view from the slide. It would be useful to add your standard deviation of your 5 readings in the table.
- Develop your own table, but please share your results in order to improve the accuracy of this short-cut.

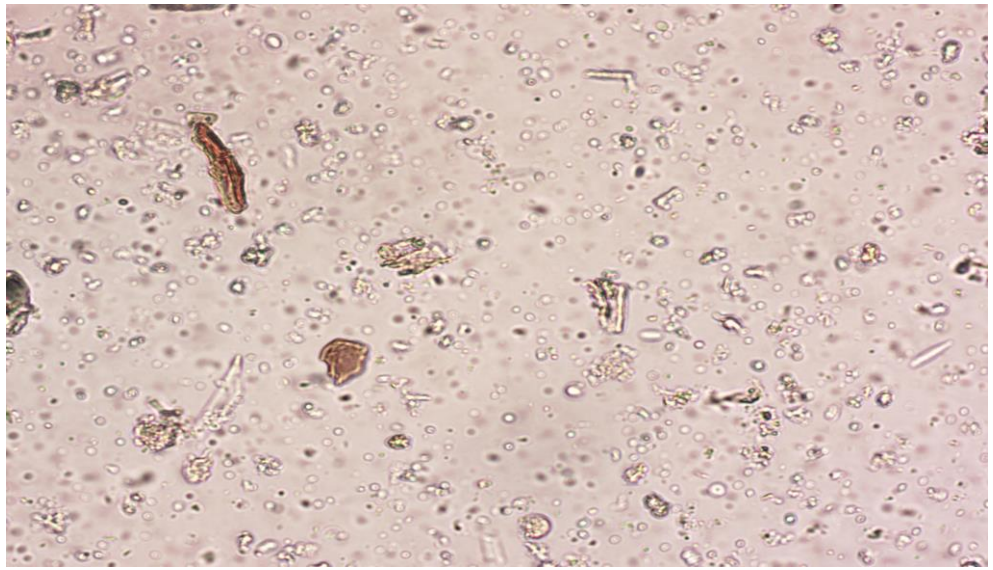
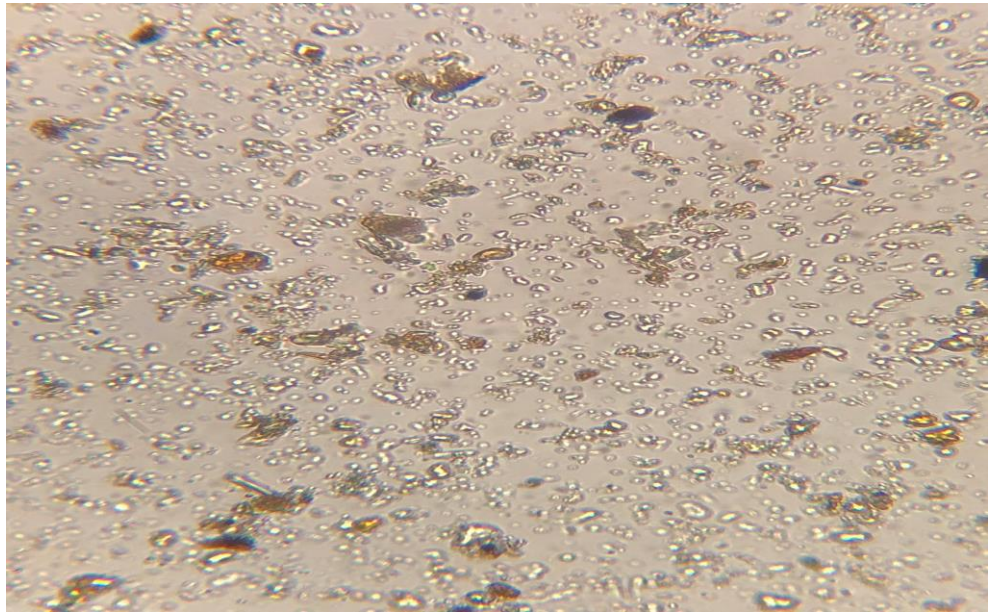
1:5 Dilution	Actual Density



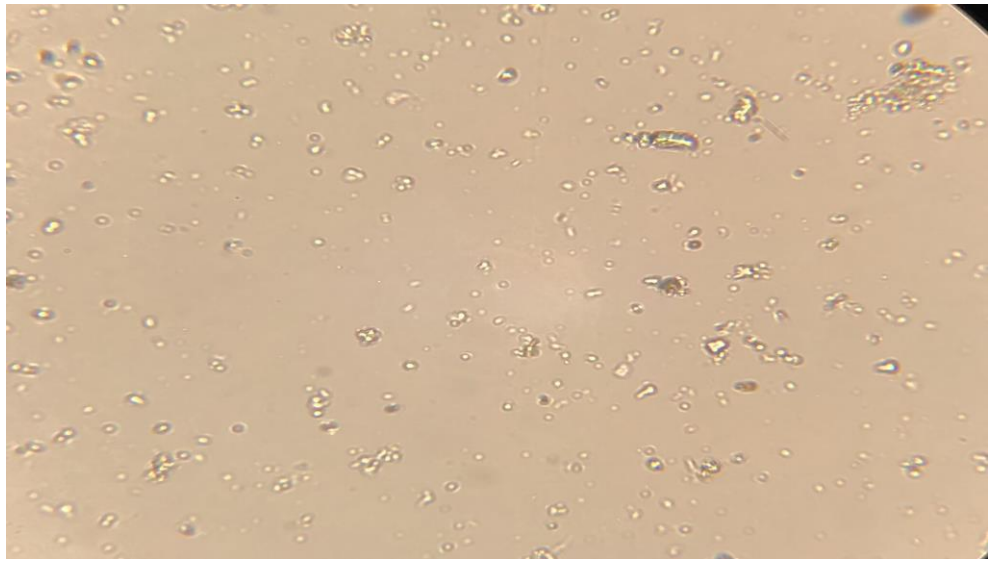
10,500 micrograms bacterial biomass per gram soil:
1:500 dilution



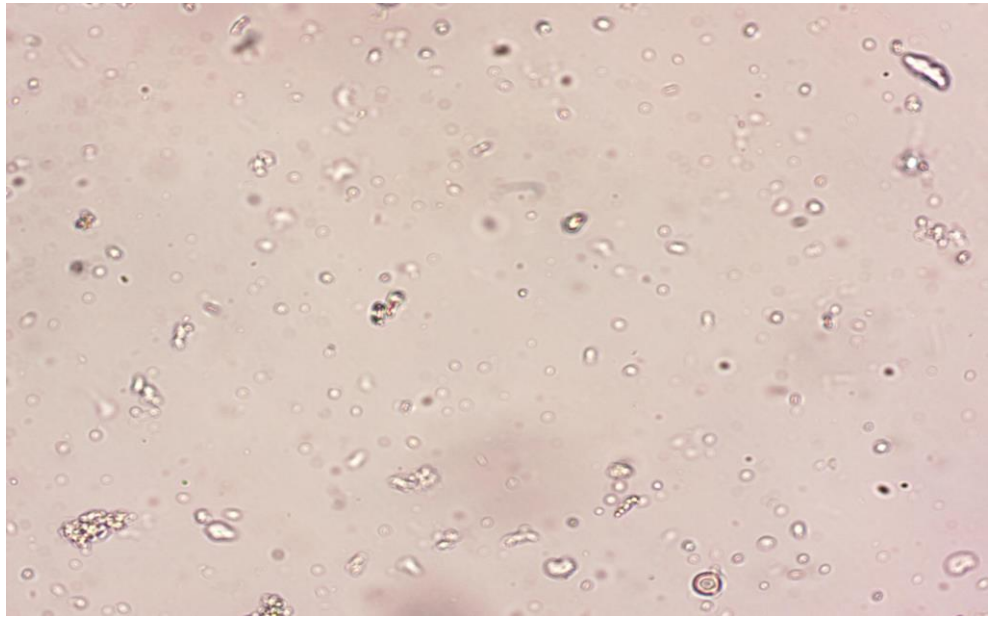
4,100 micrograms bacterial biomass per gram:
1:500 dilution

1:5 Dilution	Actual Density
	**1,860 micrograms bacterial biomass per gram soil: 1:500 dilution**
	**1,100 micrograms bacterial biomass per gram: 1:500 dilution**

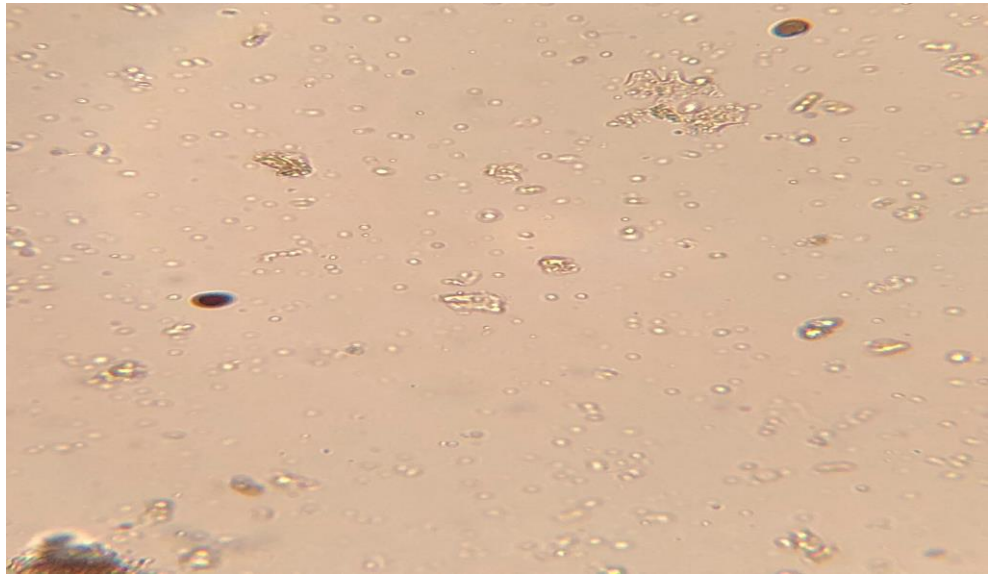
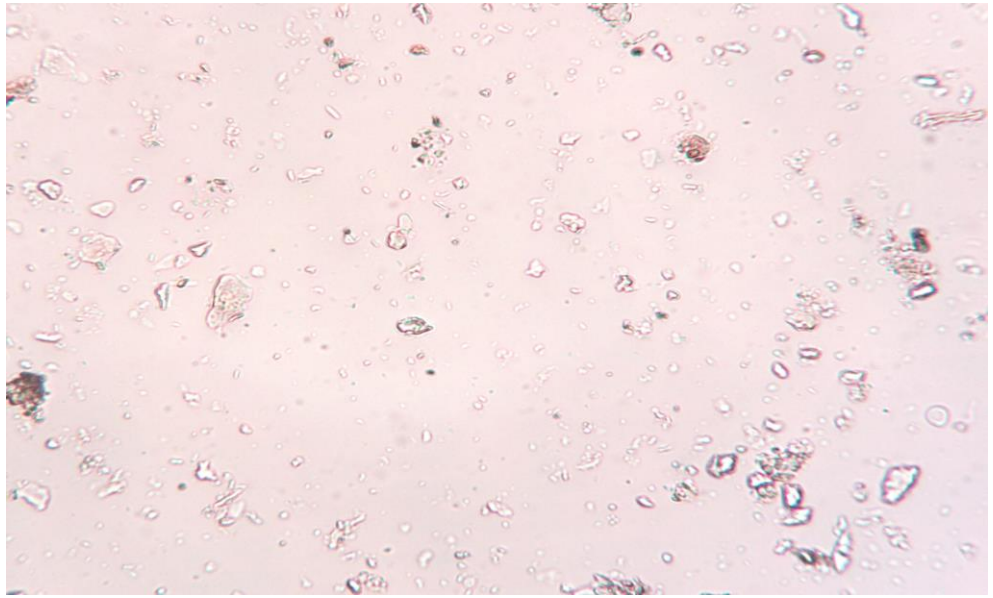
1:5 Dilution	Actual Density



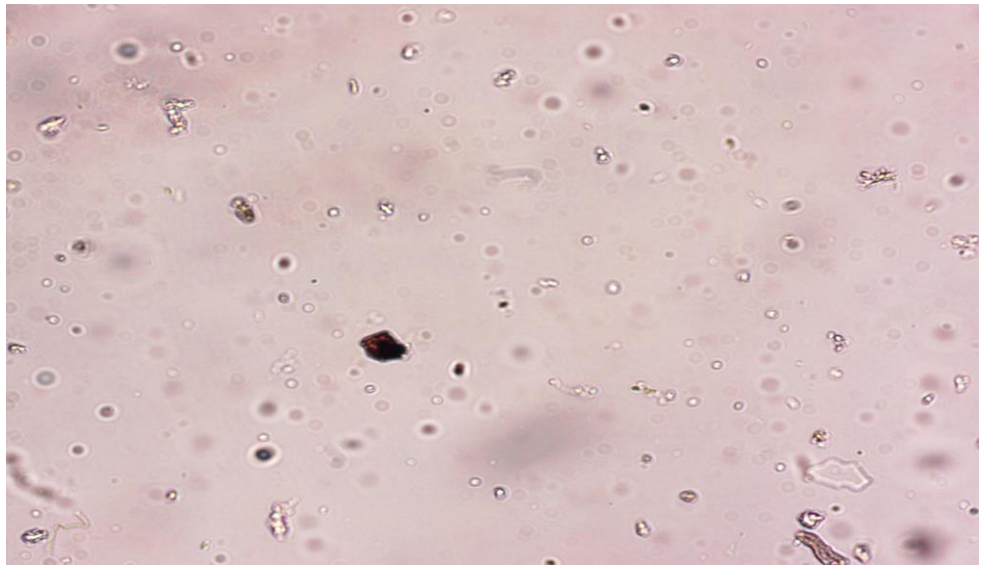
1,100 micrograms bacterial biomass per gram soil:
1:500 dilution



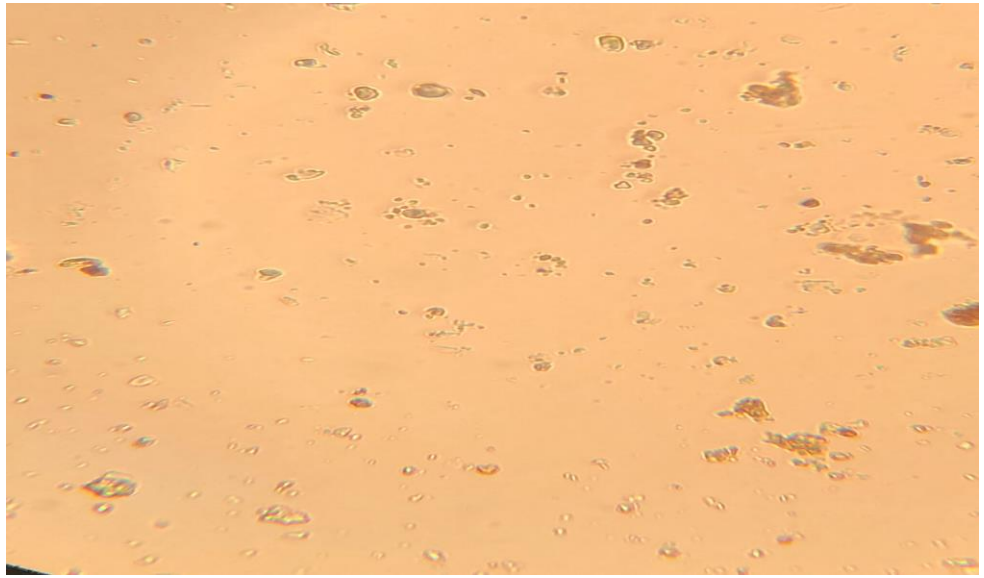
980 micrograms bacterial biomass per gram:
1:500 dilution

1:5 Dilution	Actual Density
	**580 micrograms bacterial biomass per gram soil: 1:100 dilution**
	**380 micrograms bacterial biomass per gram: 1:50 dilution**

1:5 Dilution	Actual Density



380 micrograms bacterial biomass per gram soil: 1:100 dilution



380 micrograms bacterial biomass per gram: 1:100 dilution

What can you say about this next sample?

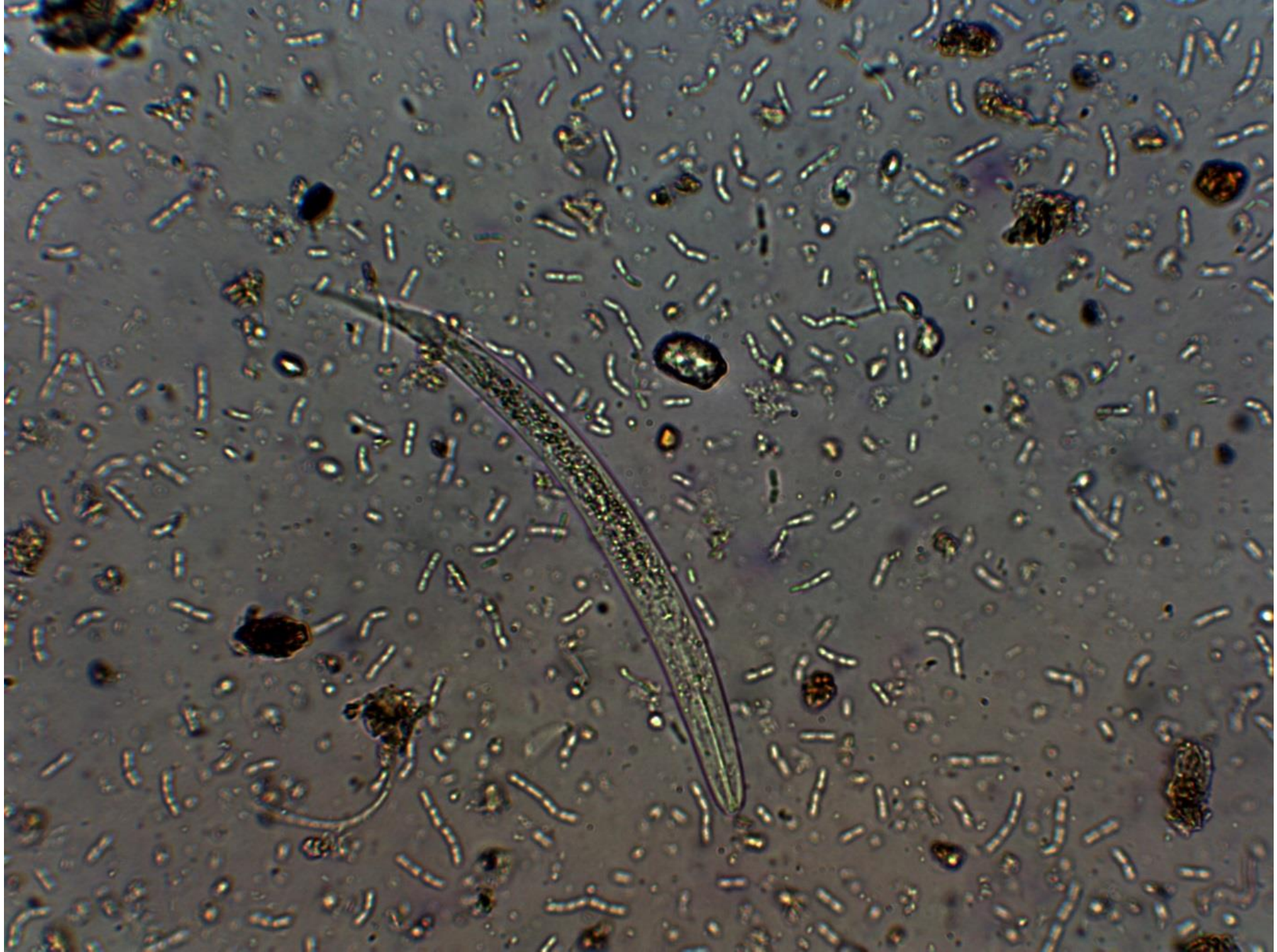
Bacteria? Actinobacteria?

Beneficial fungi / Diseases fungi (oomycetes)?

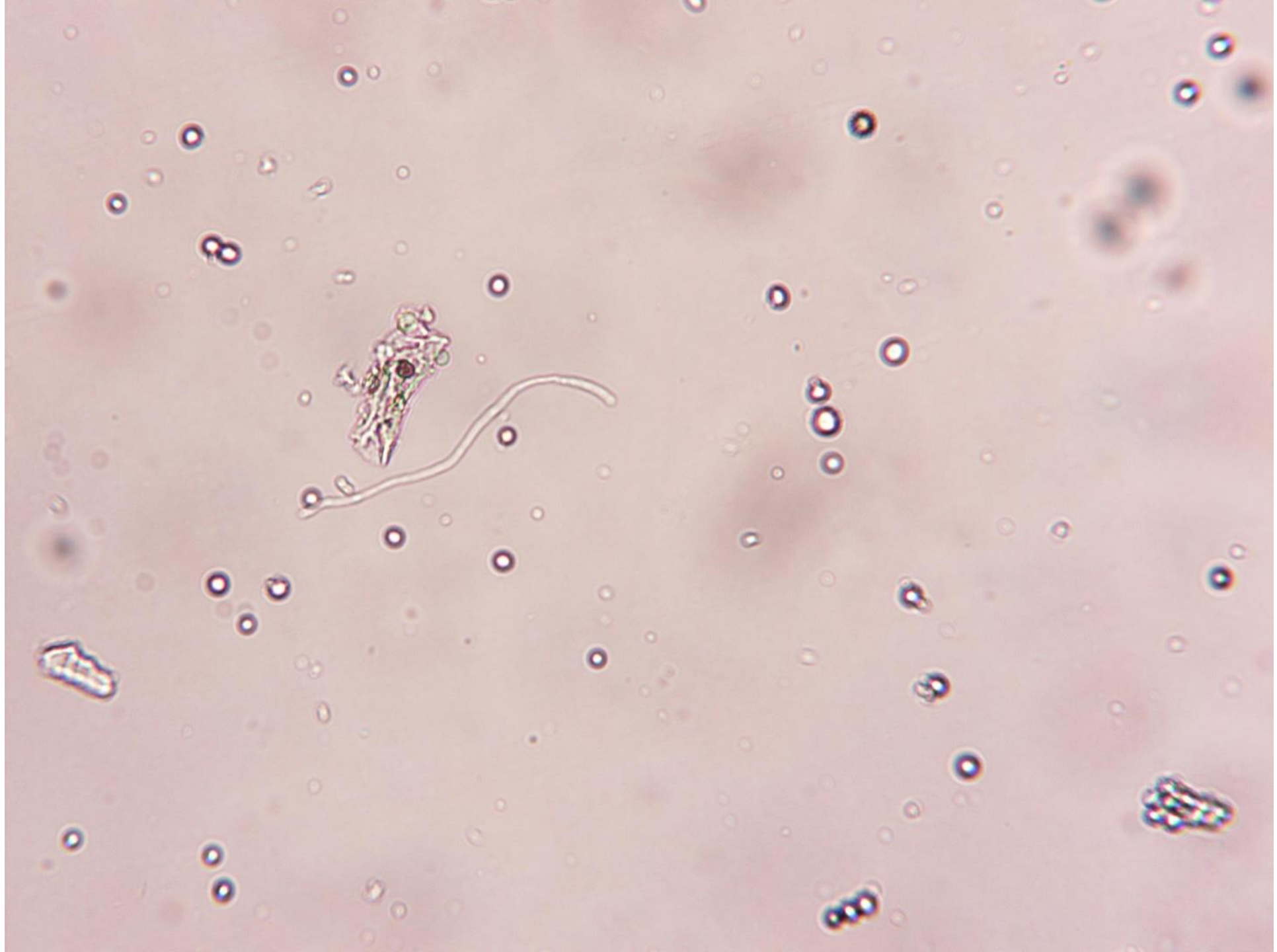
Protozoa?

Nematodes?

Aggregates? Humics? Fulvics? Background color?




Rod-shape bacteria
in a chain



Look closely at the S-shape strand on the right

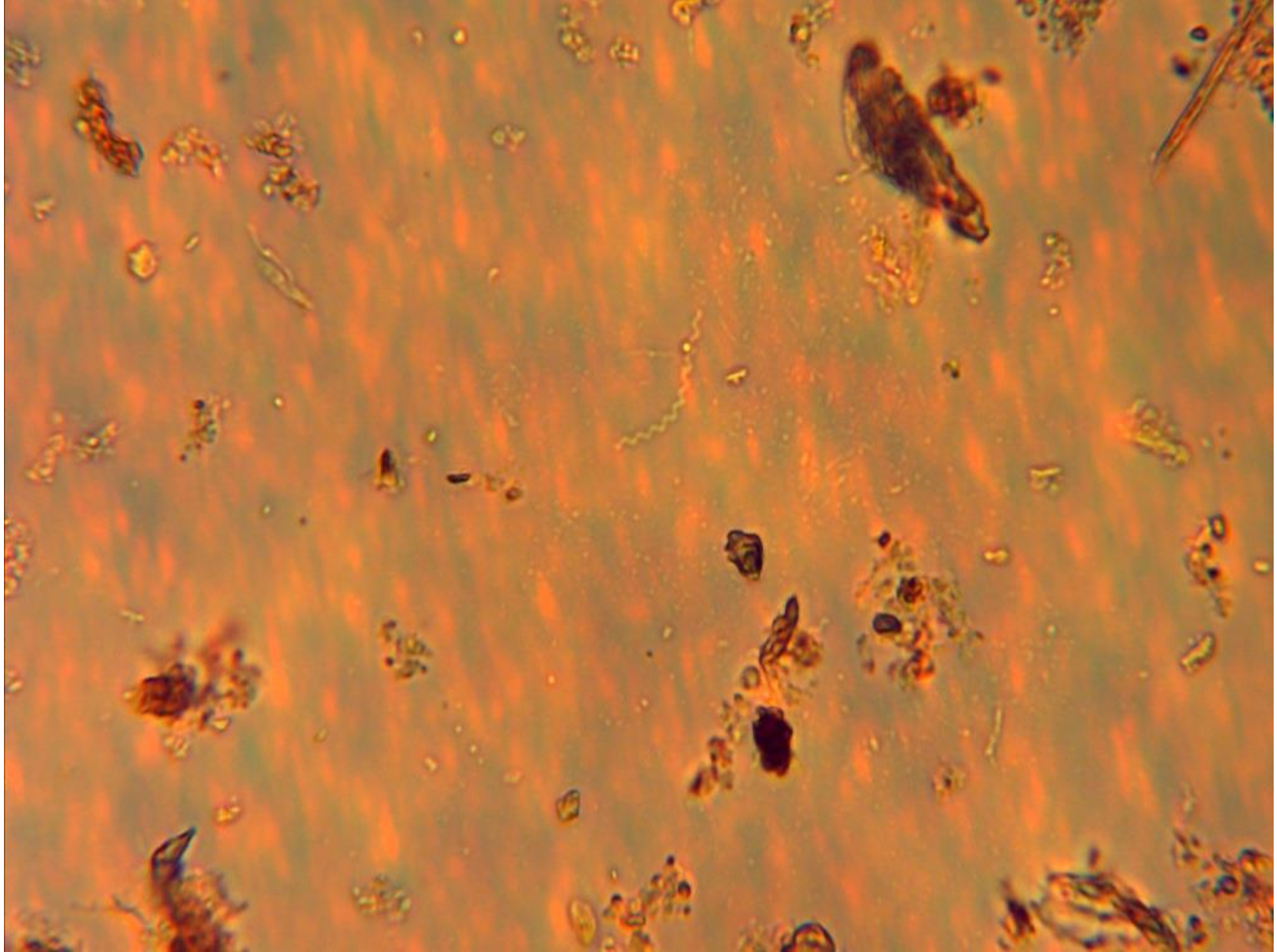
What is it?

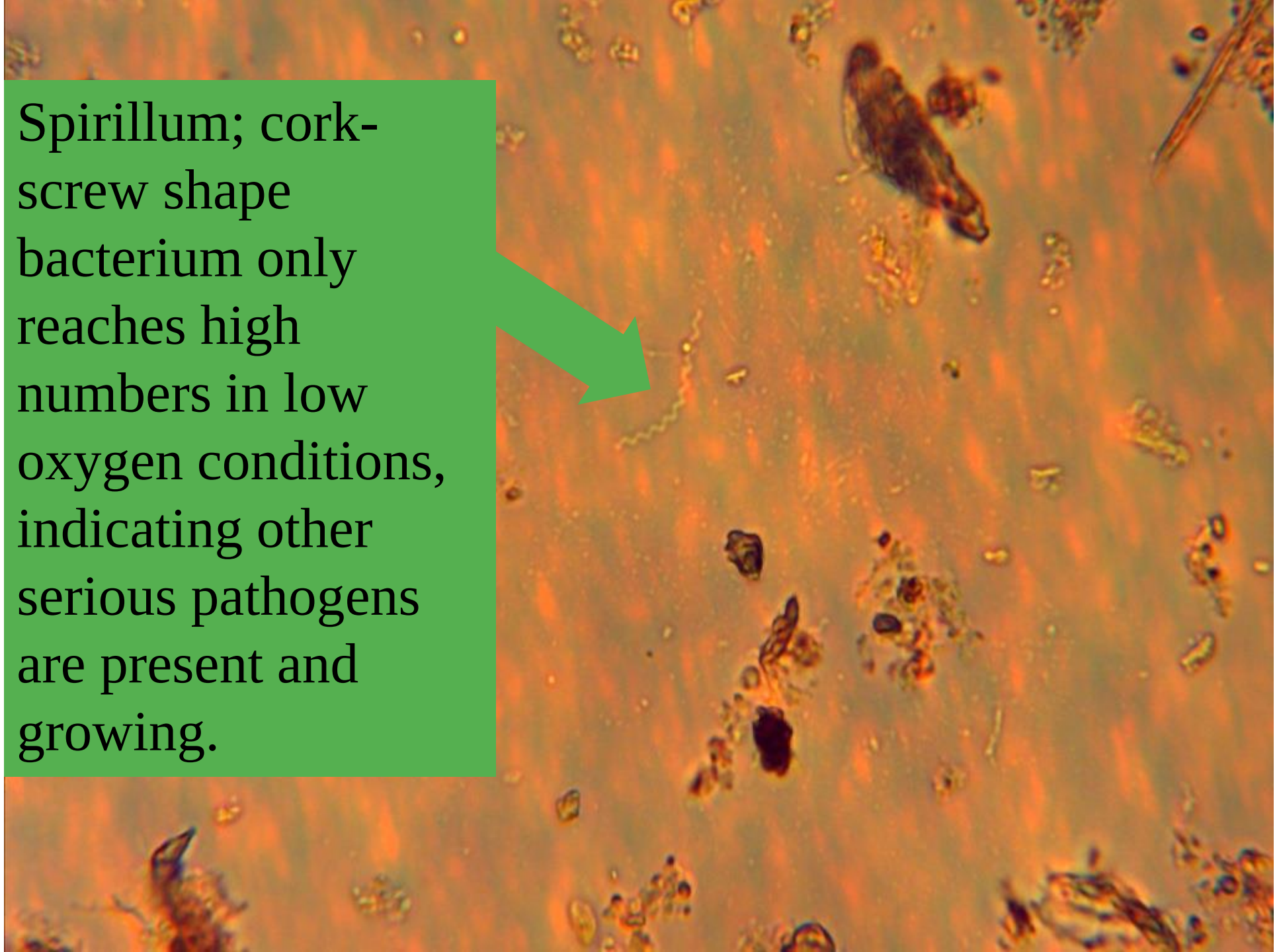
Fungal hypha? No, too slender. Fungal hyphae are at least 2 micrometers diameter, and this is only 1 micrometer wide.

Bacteria? Yes, but is it in a filament or just a chain of bacteria? If a filament is present, the outside of the chain is smooth. I can make out the individual bacteria in this chain, so NOT an actinobacteria.

Can you find the spirillum in the next picture?

- Spirillum: Think cork-screw: Spirilla spins, the waves in their bodies are stiff and do not undulate, instead they spin. Spirilla mean low oxygen conditions exist, and other serious pathogens, such Salmonella, Shigella, and Pasteurella could be growing as well.
- Make sure everything that the sample came in contact with is cleaned with alcohol, or well-washed in cleaning solutions. WASH YOUR HANDS! Re-compost the material, or layer onto a worm bin to let the worms take care of the problem.



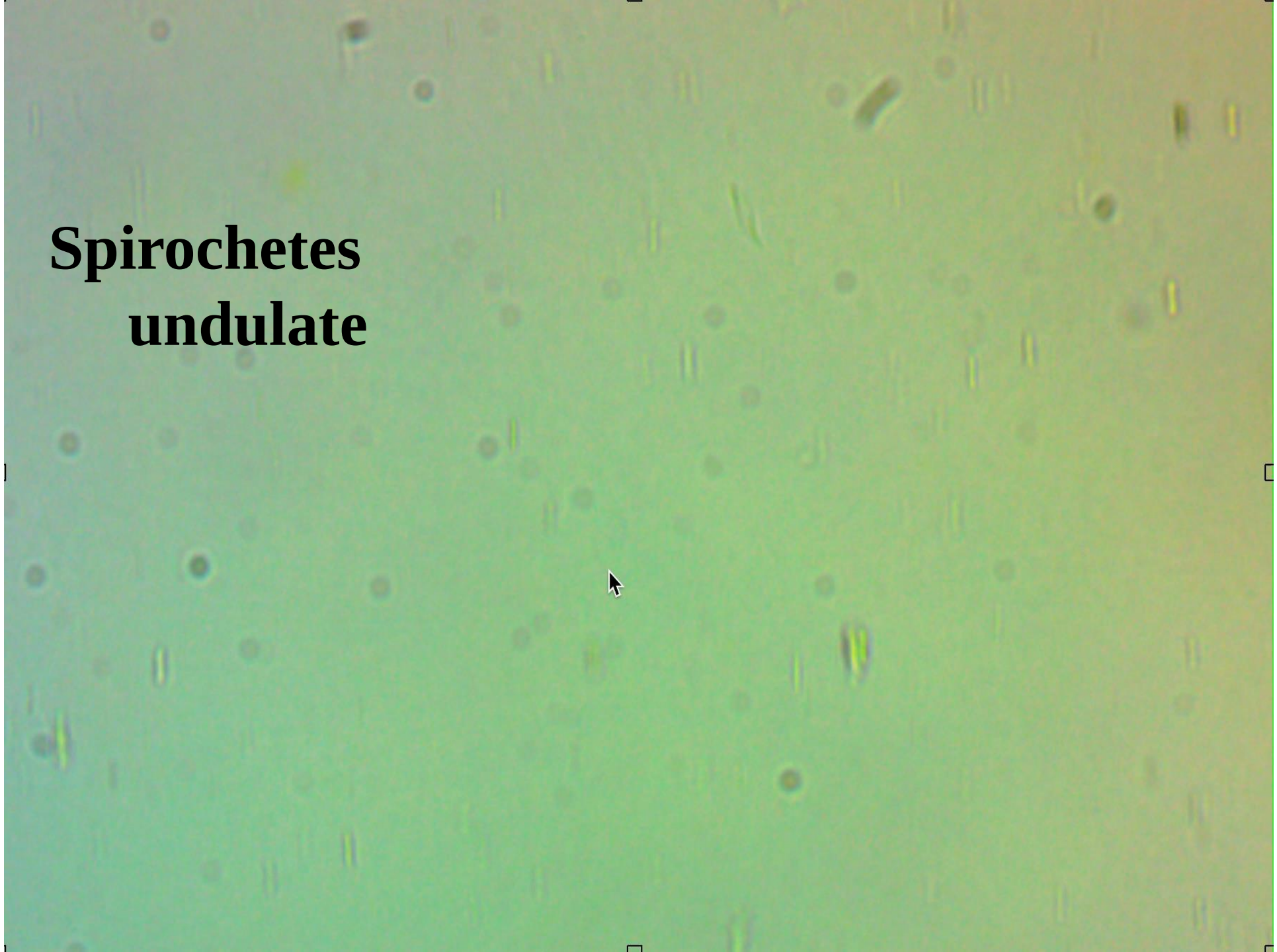

Spirillum; cork-screw shape bacterium only reaches high numbers in low oxygen conditions, indicating other serious pathogens are present and growing.

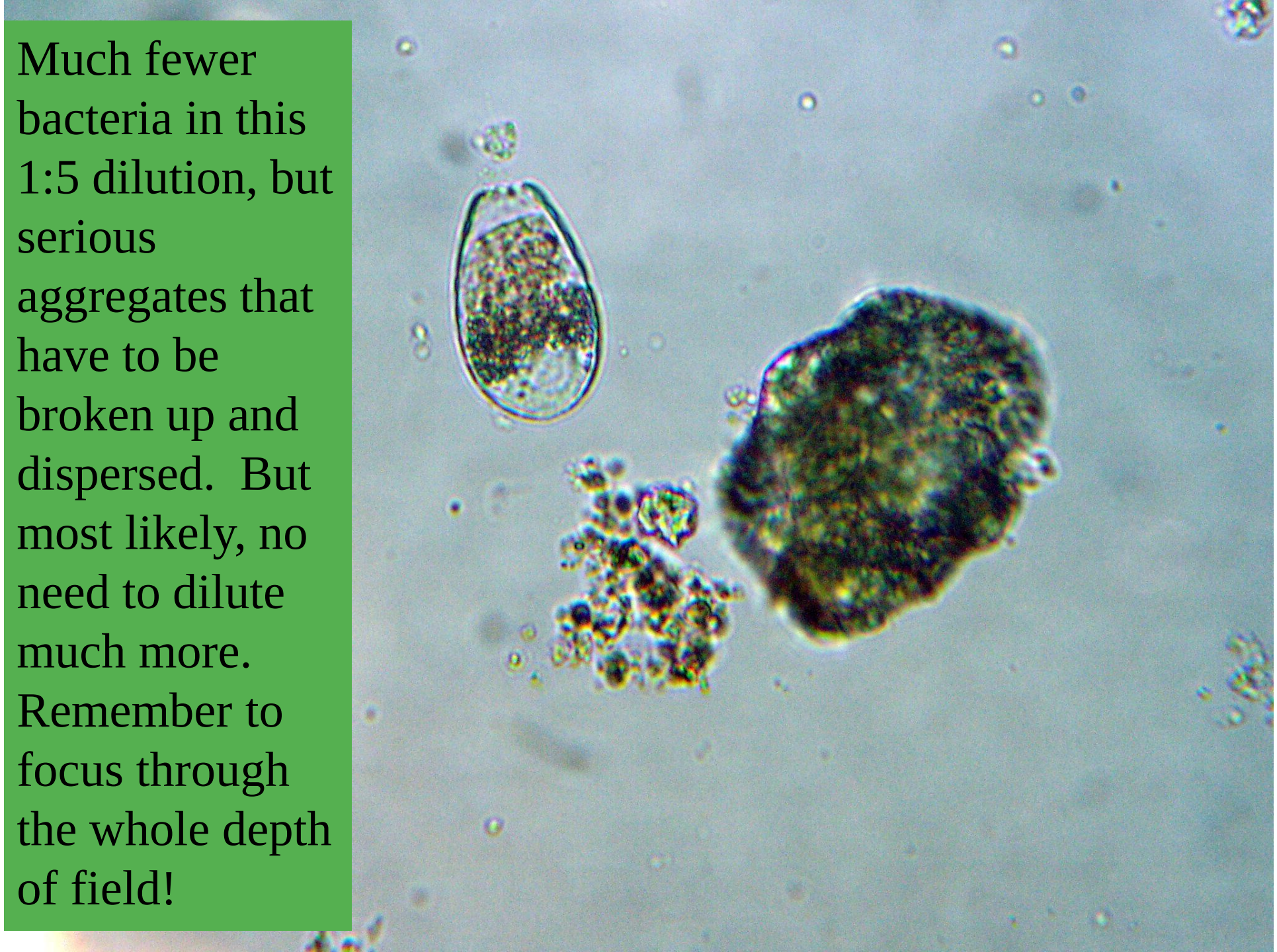
Anything else to note about the sample?

Look at the grainy background on this slide. The condenser isn't properly focused. The condenser is focused on the light source instead of the condenser.

Adjust the condenser focus knob slightly to remove that grainy background.

How about aggregates? Diversity of bacteria? Any fungi? Color of the sample itself? The color is only seen in reduced oxygen conditions. The reduced mineral that results in this color is not known.


Spirochetes
undulate



Much fewer bacteria in this 1:5 dilution, but serious aggregates that have to be broken up and dispersed. But most likely, no need to dilute much more. Remember to focus through the whole depth of field!

Anything to note about the sample?

Bacteria? Actinobacteria? Mostly just bacteria in aggregates. Note the aggregate built just by bacteria versus the aggregate glued to the sand grain.

Beneficial fungi / Diseases fungi (oomycetes). None.

Protozoa? Testate amoebae. Note the clear amoebae sitting at the far end form the opening to the test, and the aggregate of bacteria it has pulled inside the test.

Nematodes? None.

Aggregates? Humics? Fulvics? Background color?

Compost Viewed Using Scanning Electron Microscopy

The sample was glued to a stub, dried, and then a thin layer only nanograms thick of gold-palladium spread over the surface of the sample. Electrons were bombarded at the surface and the speed at which the electrons return to the detector makes the image.

At least 16 different bacterial species, 12 fungal species, including yeasts (ouch, bad guys), and a protozoan observed in the tiny amount of compost, only 50 X 40 micrometers surface area. This is good compost…….


Magn
2000x
Exp
10
10 µm
Compost 01-12-17

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)