

**Dr. Elaine's™ Soil Food Web Foundation  
Courses**

**Course Manual**

**Foundation Course 4:  
Microscopy  
Chapter 1**

*Congratulations* on completing FC3 and welcome to FC4 - Microscopy!! Learning how to efficiently and properly use your microscope will open up and bring to life the microscopic world that you have been learning about from Dr. Ingham! With this tool and skill, you will be able to assess any soil, compost, extract, or tea within minutes of collecting a sample.

In FC4 Microscopy - Chapter 1, you will learn about each of the components of the microscope, their functions, and how to use them in focusing your microscope. Dr. Ingham goes into great detail on the process of preparing a sample and the necessary steps required to bring your sample into perfect focus! FC4 builds upon the knowledge you acquired in FC1, to expand your familiarity with the different morphological characteristics of each of the soil food web microorganism groups. You will gain confidence in properly identifying each organism as you perform microscope assessments of various samples.

As with any new skill you will need to invest time in order to refine the ability to identify many different types of microorganisms.

We wish you all the best as you continue on with Foundation Course 4!

*Dr. Elaine's™ Soil Food Web team!*

## HOW TO USE THIS MANUAL

Used in conjunction with the series of online lectures, the Dr. Elaine's™ Soil Food Web Foundation Courses Manual provides the following:

- Overview of the lecture
- Lecture Contents containing Key Points that clarify and/or support information delivered in each lecture. *Please make sure you read these before watching each lecture!*
- Glossary of key words, terms and abbreviations used in each lecture, and
- References and Further Reading

**On-screen prompts:** During the online video lectures you will occasionally see information popups. These on-screen prompts will refer you to:

- Other sections of this course
- Key Points that are found in this Course Manual

**Time references:** These appear in this Course Manual:

- After **Key Points**, for example, "**Key Points: [34:18]**" - this indicates the time in the online video at which Dr. Ingham mentions a particular topic or concept. The information in the Key Points sections of the Course Manual will help you better understand these topics and concepts.

**BEFORE WATCHING EACH LECTURE YOU WILL NEED TO DO THE FOLLOWING:**

- Read through the section of the Course Manual for that lecture.

- Review the Course Glossary for the terms that are listed in the lecture.
- Review the relevant Appendices for the lecture.

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# LECTURE 1

## Lecture 1: Using a Compound Microscope for Shadowing Microscopy (Part 1) [43:06]

### 1.0 OVERVIEW

Lecture 1 begins by explaining the microscope requirements and additional equipment needed for performing biological assessments. Having a solid understanding of each component of your microscope will help in building this valuable skill of microscopy and will enable you to bring this microscopic world easily into view.

### 1.1 LECTURE CONTENTS

#### 1.1.1 Compound Microscope requirements for performing biological assessments

##### Key Point [3:28] Compound Shadowing Microscope Requirements

- **Total magnification:** 40X-100X-250X-400X; higher magnification is not needed
  - Eyepieces: wide field WF10X; higher magnification eyepieces generally decrease resolution
  - Objective lenses: Achromatic DIN 4X, 10X, 40X(S)
- **Viewing head:** 45 degrees inclined 360-degree swiveling trinocular
  - Sliding adjustable interpupillary distance: 2-3/16inch ~ 2-15/16inch (55mm ~ 75mm)
  - Ocular diopter adjustable on one of the eye tubes. Some microscopes come with 2 adjustable diopters, one on each tube, this option is acceptable but not necessary.
- **Nosepiece:** Revolving quadruple (i.e. space for 4 objective lenses)
- **Focus adjustment:** Coaxial coarse and fine knobs on both sides
- **Stage:** Mechanical double layer size: 4-1/2inch x 4-15/16inch (115mm x 125mm)
  - Stage x-y stroke (travel range): 2-13/16inch x 1-3/16inch (70mm x 30mm)
- **Condenser and diaphragm:** NA 1.25 Abbe condenser **with iris diaphragm**
- **Transmitted (lower) illuminator:** LED light, intensity adjustable
- **Power supply:** AC/DC adapter, 100V-240V (UL approved)
- 5-year warranty against manufacturing defects is recommended

#### 1.1.2 Additional equipment needed for doing a biological assessment with a Compound Microscope

##### Key Point [15:08] Additional required microscope assessment equipment

1. **Slides** - Size: 3.6 x 3.1 x 1.2 inches
2. **Coverslips** - Square Cover Glass, 18mm x 18mm Size, No. #1.5 Thickness (0.17 mm)
3. **Test tubes** - Size: 15ml, conical bottom, plastic, with screw top lid. Make certain the tubes ordered have the 1 ml mark.
4. **Transfer Pipettes** - Karter Scientific 206H2 - 3ml Graduated Transfer Pipettes, Vol 7ml, 1/2ml Graduations, 155mm
5. **Gosky Universal Mount** for holding cell phone in position for taking microscope picture;  
[https://www.amazon.com/gp/product/B013D2UL06/ref=oh\\_aui\\_search\\_detailpage?ie=UTF8&p\\_sc=1](https://www.amazon.com/gp/product/B013D2UL06/ref=oh_aui_search_detailpage?ie=UTF8&p_sc=1)

1.1.3 **Key Point [18:34]** Correction: Dr. Ingham mentions that she wants you to use a #1.5 thick coverslip for biological assessments. This coverslip size actually has a thickness of 0.17 mm or 170  $\mu\text{m}$ , and not 10  $\mu\text{m}$  as is accidentally stated in the lecture.

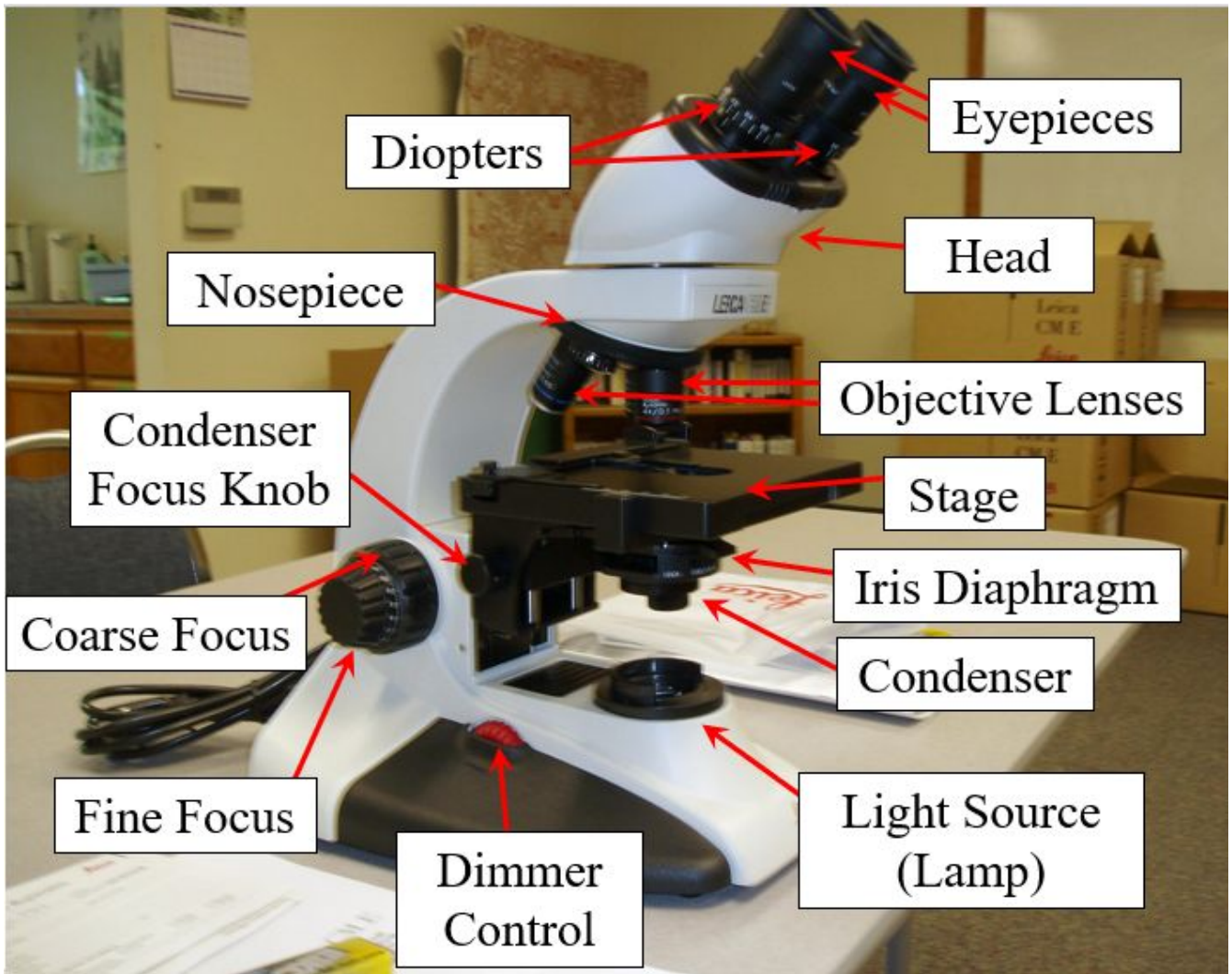
1.1.4 Recommendations for microscope cleaning and storage

- ALWAYS remember to place the dust cover on the microscope when it is not in use.
- Dirt and dust get into the stage gears and focusing ability becomes difficult.
- Dust gets into the internal lenses of the microscope and you should not attempt to remove that dust. Let a professional do it.

1.1.5 Compound Microscope parts breakdown

**Key Point [37:59]**

**Figure 1**



1. **Light Source (Lamp):** Plug in the microscope. Turn the lamp (bottom of the microscope) on. Adjust the dimmer control to regulate the brightness to a comfortable intensity.
2. **Dimmer Control:** Controls the brightness/intensity of the light source (lamp).
3. **Eyepieces:** When viewing through the eyepieces, there should be  $\frac{1}{4}$  -  $\frac{1}{2}$  inch between your eyes and the lenses. Adjust the interpupillary distance just as you would with a pair of binoculars. A single round field of view should be visible once the eyepieces have been properly adjusted to fit your interpupillary distance. You may need to move your eyes farther from or closer to the lenses as you adjust the distance between the eyepieces until you see one circle of light.
4. **Diopters:** Focussing both eyepieces to the same depth is critical in order to avoid very painful headaches. Some microscopes will have diopter adjustment rings on one eyepiece, and some will have diopters on both eyepieces. In both situations, you will want one diopter to be stationary. Bring this eyepiece into focus first using the fine focus knob, while peering through the stationary eyepiece, only (i.e., close the eye not peering through this eyepiece). Next you need to focus the non-stationary eyepiece by adjusting the diopter on that eyepiece, while peering with the corresponding eye only, bringing this non-stationary eyepiece into focus. Your eyepieces are now adjusted so that your eyes are focused on the same focal plane. Because the focal depth of most people's eyes are not on the same plane, the discrepancy between the eyes must be compensated by adjusting the diopters whenever there is a change in user. *Reference Lecture 2.1.7.*
5. **Headpiece:** The headpiece connects the eyepieces to the main body of the microscope. Many models include an additional viewing tube for camera placement.
6. **Nosepiece:** The revolving nosepiece on a microscope houses the objective lenses.
7. **Objective Lenses:** Only three objective lenses are needed for this work; a 4X scanning lens, a 10X lens, and a 40X working lens. Most measurements are performed using the 40X objective. But to start, move the stage to its lowest point, then dial the 4X lens into alignment with the sample. Now you can safely move the stage up, using the coarse focus knob. There are coarse focus knobs on both sides of the microscope. Care must always be taken not to dial-in an objective that may come into contact with the sample, as this will likely result in permanent damage to the objective lenses, which can be very expensive to replace. *Reference Lecture 2.1.4.*
8. **Stage:** The flat platform where the slide is placed. The movement of the stage in the x-y plane is controlled by 2 dials on a knob, called the Stage Control, *reference Lecture 2.1.6, Figure 6.*
9. **Coarse Focus:** Initially use the Coarse Focus knob to bring the specimen into general focus. The objects on the slide will be somewhat focused but will need the Fine Focus to bring objects into sharp focus.
10. **Fine Focus:** Fine tunes the focus and increases the detail of the specimen. After using the Course Focus, use the Fine Focus to bring objects into sharp focus. Use the Fine Focus when bringing objects into focus that are in different planes within the sample depth or when focusing between Objective Magnifications.
11. **Iris diaphragm:** Adjusts the amount of light that reaches the specimen. Important for improving shadowing and visibility of microorganisms. *Reference Lecture 2.1.6.*

12. **Condenser:** Gathers and focuses light from the light source (lamp) onto the specimen being viewed. A focused Condenser will give a sample a clear background. *Reference Lecture 2.1.5.*
13. **Condenser Focus Knob:** Moves condenser up and down to focus the light on the specimen.

## 1.2 GLOSSARY TERMS *(Please refer to the glossary on page 41 )*

The following glossary terms are used in this lecture: **Aberrant; Assess/Assessing; Compound Microscope; Shadowing.**

# LECTURE 2

## Lecture 2: Using a Compound Microscope for Shadowing Microscopy (Part 2) [55:01]

### 2.0 OVERVIEW

Lecture 2 continues with in-depth explanations of the processes of preparing a sample and focusing the microscope.

### 2.1 LECTURE CONTENTS

2.1.1 Preparing and diluting a solid sample for a biological assessment

2.1.2 Instructions for using a pipette, microscope slide, and coverslip when preparing a sample

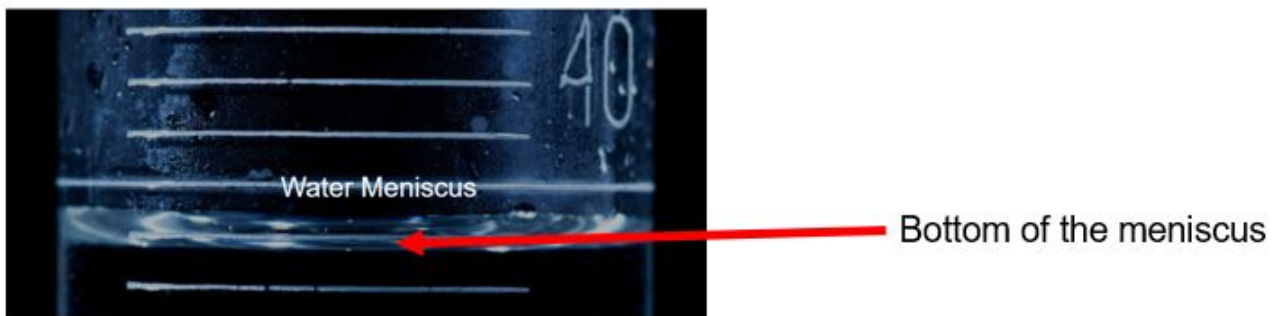
#### Key Point [6:47]

Instructions on calibrating a pipette (or dropper) to ensure accuracy in biological assessment equations. First, gather supplies needed: pipette/dropper, clean water, and either a test tube with clearly marked (1ml) delineations or a digital scale that is accurate to  $\pm 0.1$  g.

#### Volume Method (Test tube)

If calibrating using the volume method with a test tube, most will have a clear 2 ml mark. Draw the pipette full of water; count the number of drops required to fill a dry test tube to the 2 ml mark, drop by drop. To insure an accurate drop count, make sure that the pipette is held vertically and that ALL of the drops land in the bottom of the test tube, not on the walls. The bottom of the meniscus should be at the 2 ml line. The meniscus is formed due to the surface tension of water and water's interaction with a solid surface (test tube walls, in this case) and results in a 'smiley-face mouth' shape being formed by the water. The bottom of the smile is the bottom of the meniscus.

Figure 2



Ref.:

[https://www.usgs.gov/special-topic/water-science-school/science/water-meniscus?qt-science\\_center\\_objects=0#qt-science\\_center\\_objects](https://www.usgs.gov/special-topic/water-science-school/science/water-meniscus?qt-science_center_objects=0#qt-science_center_objects)

Since we filled the test tube to the 2 ml mark and would like to know the number of drops in 1 ml, the number of drops counted should be divided by two. If your test-tube has a 1ml mark you can just fill up-to 1ml and not divide the result (number of drops) by two.

If a weighing scale is used to calibrate the pipette, count the number of drops required to yield 1 gram of water. The mass of water in 1 cubic centimeter (or 1 milliliter of water), is 1 gram, i.e.

**For water:  $1\text{cm}^3$  (or 1ml) = 1g**

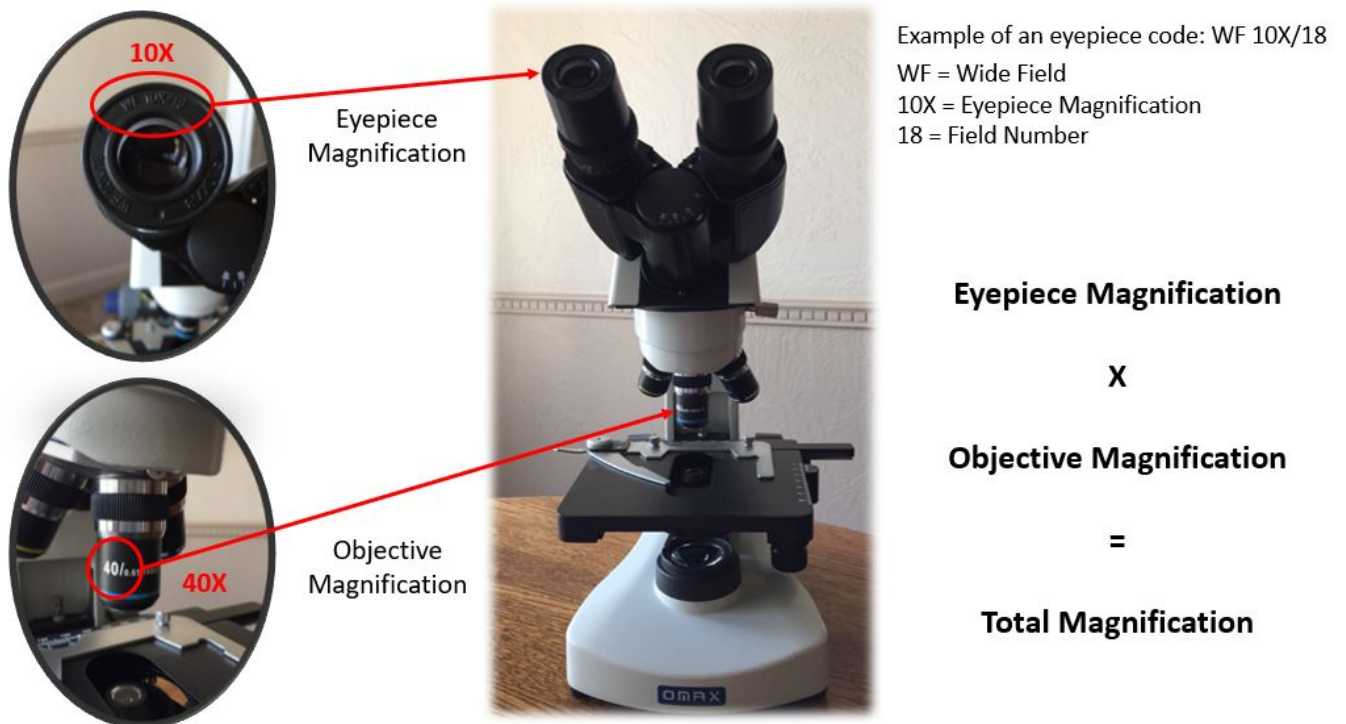
Determining the correct calibration of your pipette is vital because it determines the volume of sample under the cover slip. This volume is factored into each equation for calculating the biomass and numbers of the soil food web organisms. This number will be used in the spreadsheet or App when performing counts.

2.1.3 Explanation on the importance of the water that is used when preparing a sample and how to complex any chlorine and chloramine present in the water.

2.1.4 Instruction on focusing the Objectives

**Key Point [18:32] Locating and Calculating Microscope Magnifications**

**Figure 3:  $10X \times 40X = 400X$  Total Magnification**

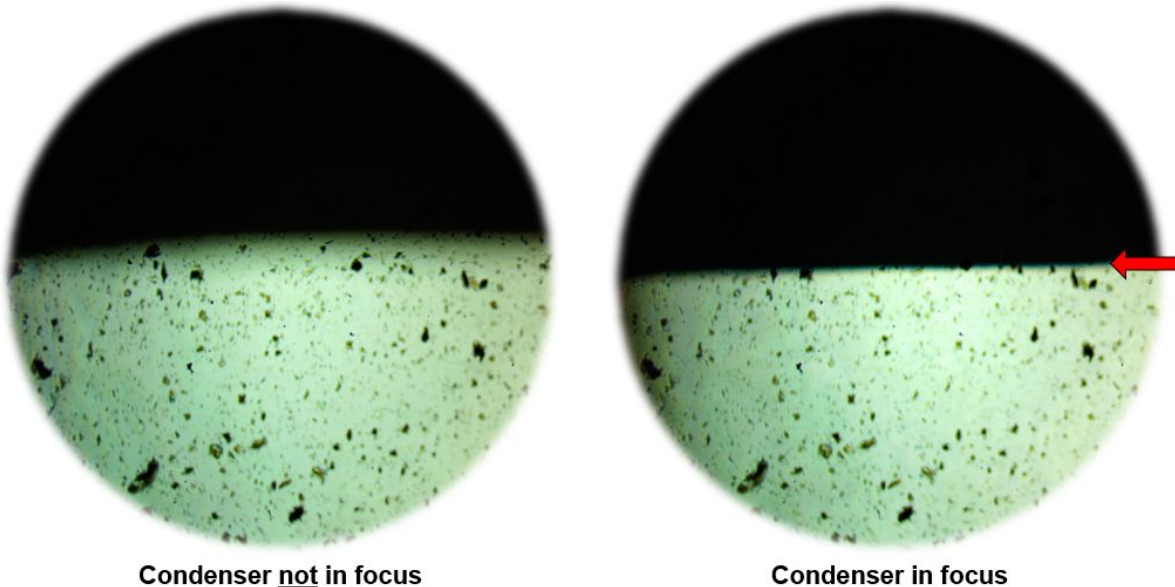


## 2.1.5 Instruction on focusing the Condenser

**Key Point [27:54]** Comparison between a condenser that is out of focus and one that is in focus.

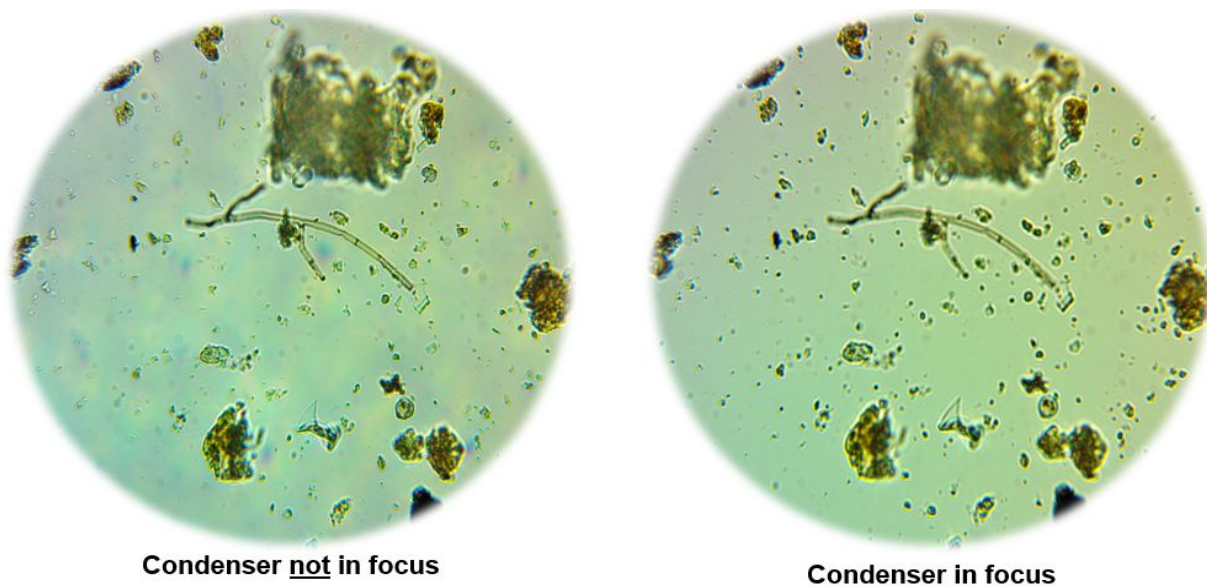
When focusing the condenser, make sure that your piece of paper is pressed tightly against the glass surface of the light source. Start by bringing the condenser to the highest point then focus it down until there is a sharp line where the papers edge is, often there will also appear to be a slight blue hue along the papers edge when properly focused.

**Figure 4**



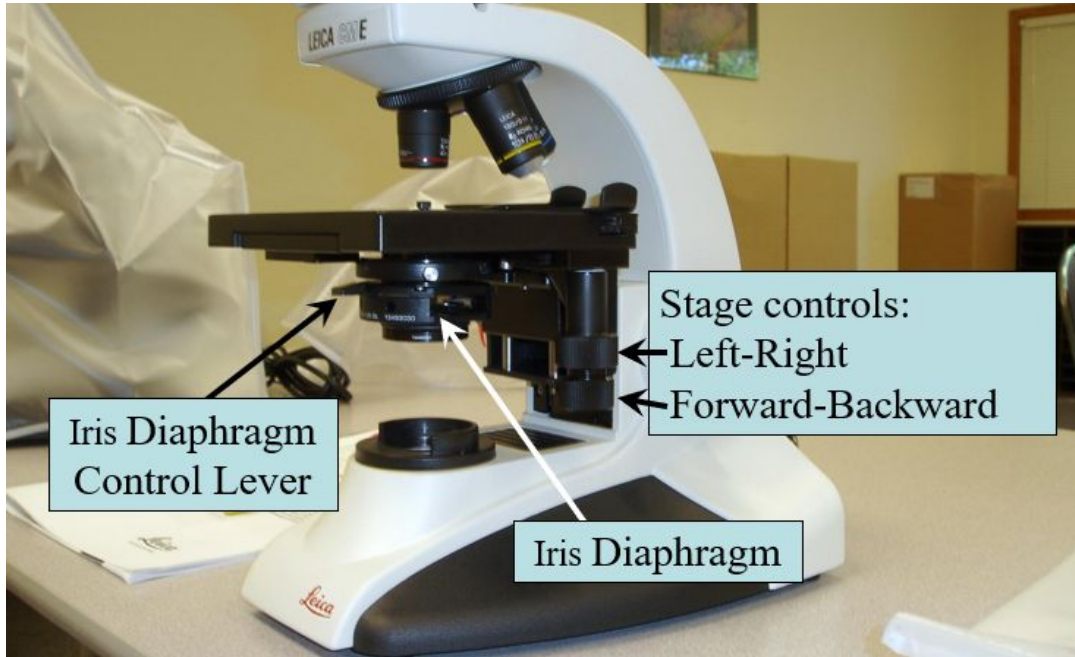
Once the condenser is focused, you can confirm this by how the background appears. If the condenser was not properly focused, then the background will appear slightly marbled with different hues of colors. If properly focused, the background will be clear.

**Figure 5** - note the background is clearer with the condenser in focus.



## 2.1.6 Instruction on proper Shadowing

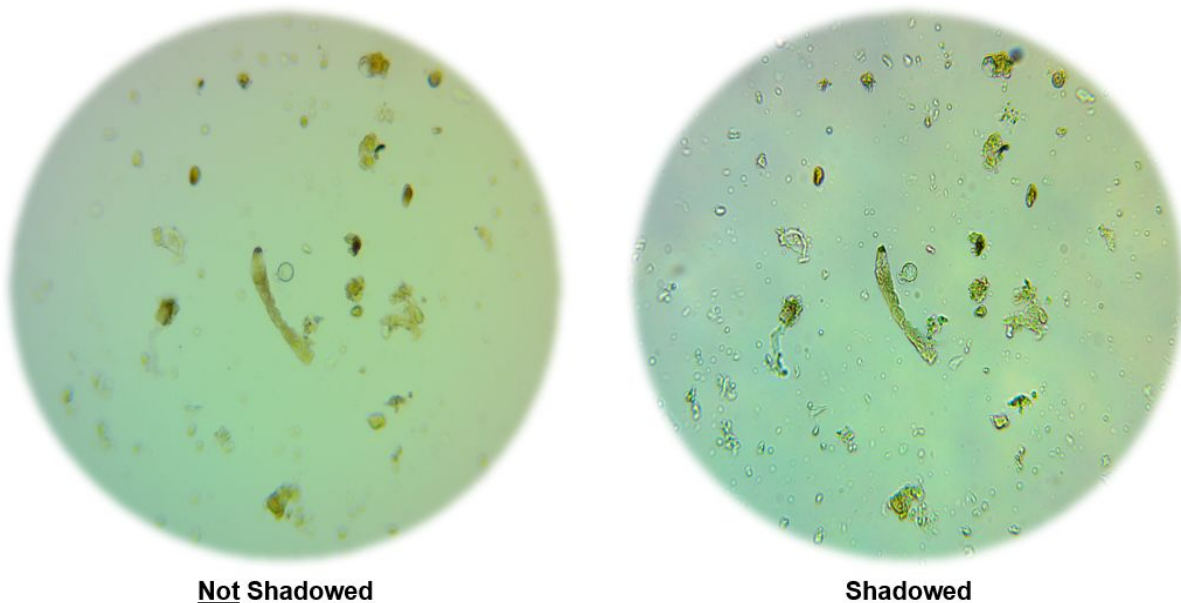
**Figure 6**



**Key Point [28:53]** Comparison image demonstrating correct shadowing

Notice the difference between the amount of organisms and materials in the image that is not correctly shadowed, versus the one that is correctly shadowed. Use the lever on the iris diaphragm to control the amount of shadowing. At one extreme, the lever will completely open the iris diaphragm allowing the light to pass through without creating any shadow. While at the other extreme, the lever will close the iris diaphragm casting a shadow onto the microorganisms in the sample, thus enabling you to view more microorganisms and with greater clarity.

**Figure 7**



## 2.1.7 Instruction on focusing the Diopters

**Key Point [33:05]** Comparison image of an unfocused Diopter versus a focused one.

Some microscopes only have one diopter, others have two diopters.

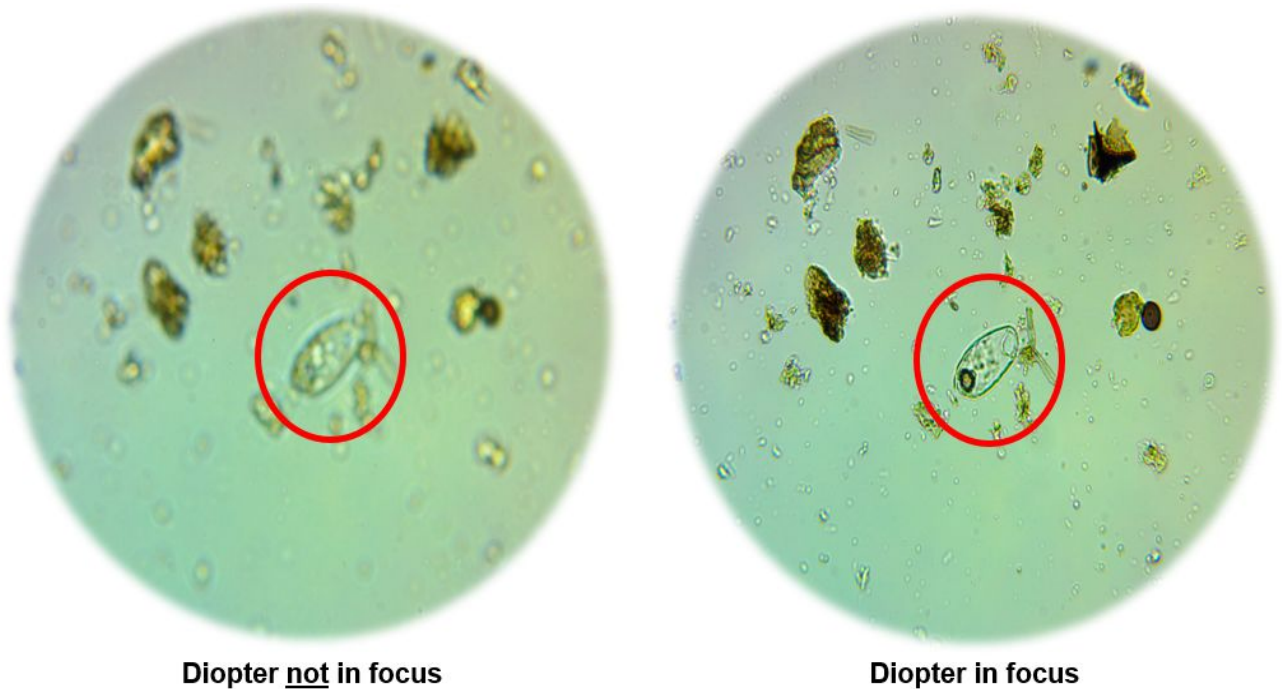
### Single Diopter Microscopes:

When focusing a diopter, find an object with crisp, sharp edges in the center of the FoV to use as a comparison image between both eyepieces. Start by bringing one of the eyepieces into focus using the fine focus knob - you will need to have the other eye closed during this step. Once the first eyepiece is focused, close that eye and open the opposite eye to focus the diopter on the second eyepiece to bring the same object into the same sharp focus as the first eyepiece.

### Microscope with two Diopters:

The method for focusing this type of microscope is discussed in the lecture.

**Figure 8**



## 2.1.8 Microscope Filters

## 2.1.9 Biological assessment - process of observations outlined

## 2.1.10 Comparison images of magnifications, i.e. 40X Total Magnification vs 100X Total Magnification vs 400X Total Magnification

## 2.2 GLOSSARY TERMS (Please refer to the glossary on page 41)

The following glossary terms are used in this lecture: **Diffuse/Diffused; Diffraction/Diffracted; Interpupillary Distance (IPD); Köhler Illumination; Resolution; Rotylenchus.**

# LECTURE 3

## Lecture 3: Bacterial Morphology (Part 1) [46:06]

### 3.0 OVERVIEW

In Lecture 3 Dr. Ingham reviews the bacterial morphology covered in FC1 and continues to cover in greater detail the identifying characteristics of bacteria. The calculation for determining the end dilution in a dilution series is explained in this lecture. In addition, this lecture covers the equation used to calculate the number of Fields of View (FoV) within the area of the Coverslip.

### 3.1 LECTURE CONTENTS

- 3.1.1 Identification is based on morphology. It is not necessary, when using Dr. Elaine's™ method, to identify to species level.
- 3.1.2 General Bacterial Classification

#### Key Point [5:14]

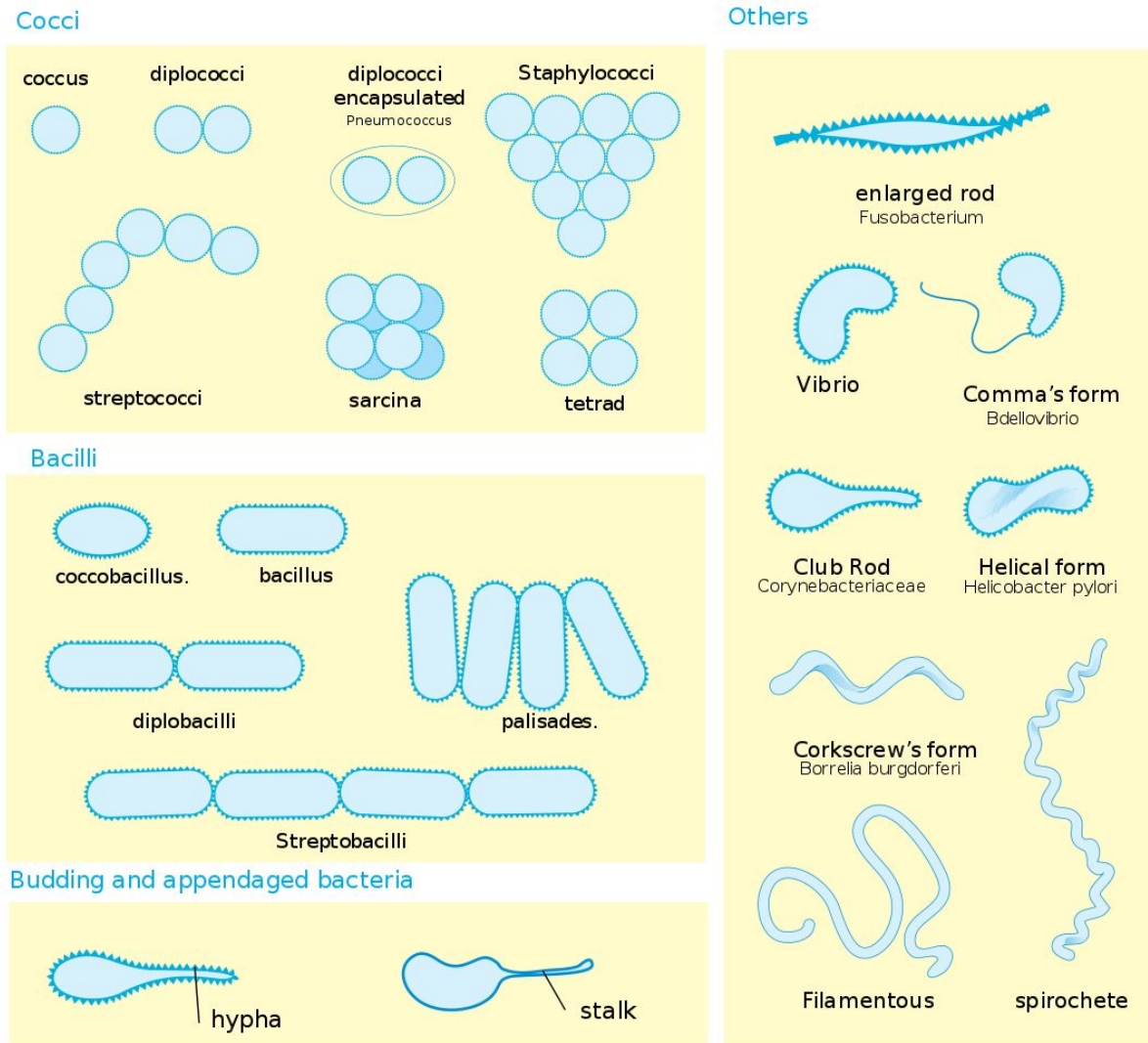
Table 1

SINGULAR	PLURAL
coccus	cocci
bacillus	bacilli
coccobacillus	coccobacilli
streptococcus	streptococci
lactobacillus	lactobacilli
vibrio	vibrios
spirillum	spirilla
spirochete	spirochetes
diplococcus	diplococci
staphylococcus	staphylococci
diplobacillus	diplobacilli
streptobacillus	streptobacilli

### 3.1.3 Bacterial Shapes

#### Key Point [18:36] Chart of Bacterial Shapes

Figure 9



Source: [https://en.wikipedia.org/wiki/File:Bacterial\\_morphology\\_diagram.svg](https://en.wikipedia.org/wiki/File:Bacterial_morphology_diagram.svg)

### 3.1.4 Distinguishing diversity in a sample based on morphology

### 3.1.6 Explanation of dilutions

#### Key Point [40:28] - Dilution Factor

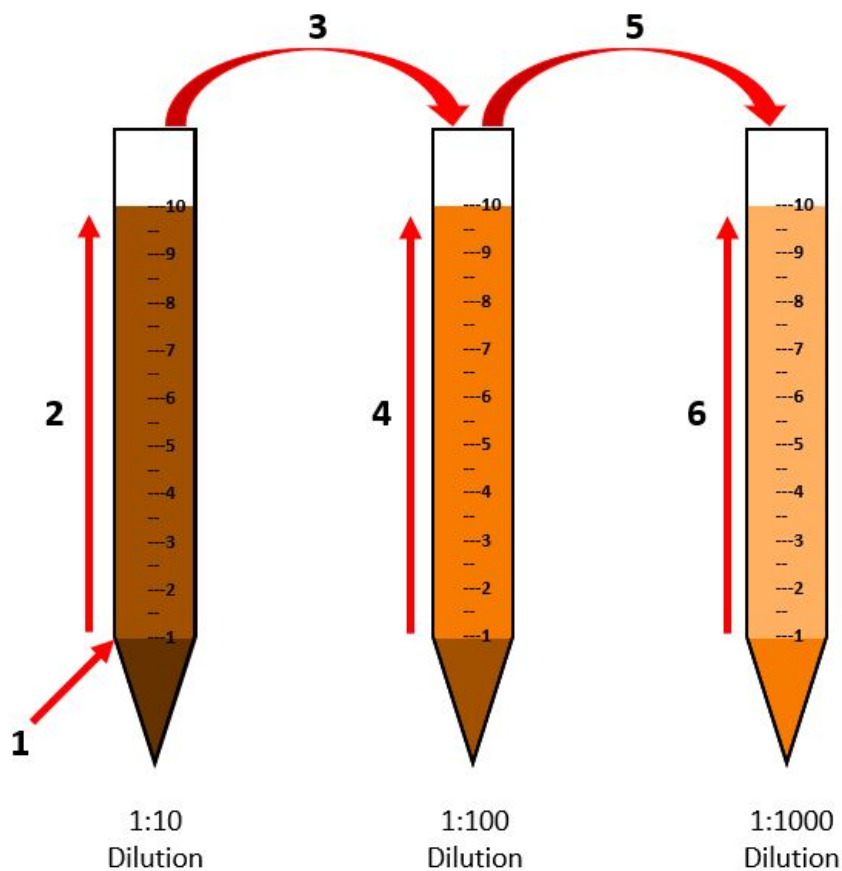
A dilution factor is the ratio of the Initial Volume to the Final Volume or  $V_i : V_f$

Example: If you take 1ml of a sample and add 9ml of water, the Dilution Factor will be  $1 : (1+9) = 1:10$ .

The process of doing dilutions is very simple once practiced a few times.

Below is an example of diluting to a 1:1000 dilution. Followed by instructions on the procedure for performing the main dilution and bacterial dilutions.

Figure 10



**Step 1:** In the first stage of this dilution, 1ml of sample is placed in the test-tube and 9ml of water are added, creating the 1:10 dilution.

**Step 2:** Then 1ml of the 1:10 dilution is taken and placed in a dry test-tube. 9ml are added to this, creating a 1:100ml dilution ( $10 \times 10$ ).

**Step 3:** A repetition of step 2, results in a 1:1000 dilution.

### **Main Dilution Instructions:**

1. Fill a test tube to the 1 ml mark with material to be assessed, e.g. soil, compost, extract, tea.
  - a. Often the main assessments for extracts and teas will be done at a 1:1 dilution; which means that no dilution of the sample material is necessary and a well mixed sample drop can be put directly onto a microscope slide from the sample source.
2. Add water to the test-tube upto the 5ml mark. Shake sample for 30 seconds.
  - a. This is a 1:5 Dilution. This is the dilution at which most initial microorganism observations will be completed, i.e. nematode, actinobacteria, fungi, oomycetes, and protozoa. Occasionally, if visibility is poor due to high levels of debris (e.g., organic matter), clay particles, or the high density of microorganisms, you may need to dilute further.
  - b. On occasion, you may need to use dilutions of 1:2, 1:3, or 1:4 when performing extract or tea assessments so that you can better observe and count the microorganisms, based on the density of the material being observed.
3. Observe the sample at the 1:5 dilution to assess whether this is a suitable dilution for the sample. If so, proceed to perform the biological assessment at this dilution. *Reference Lecture 7.1.4*
  - a. It is important that there is a consistent time between when the sample shaking has been completed to when the pipette is used to take the sample. Around 10 seconds is a suitable period of time. The important thing here is that you always wait for the same amount of time, as any variation will distort your results.
4. If a biological assessment can not be completed at the 1:5 dilution, then the sample will need to be diluted further until the dilution rate will allow observation of the sample. Follow the dilution factor equation until the sample has good visibility of the microorganisms.
  - a. The common dilution sequence when going to a higher dilution is - 1:10, 1:50, 1:100, 1:500, 1:1000.

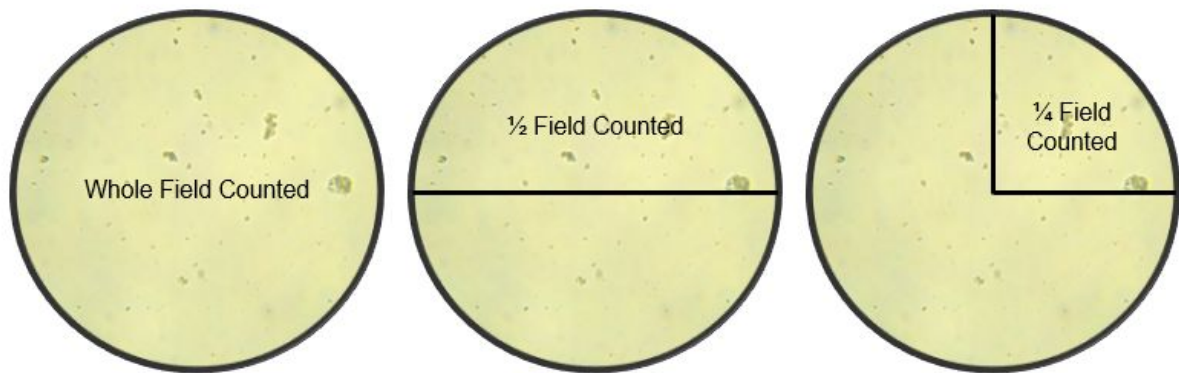
### **Bacterial Dilution Instructions:**

1. If it's necessary to dilute to a higher dilution factor in order to perform a bacterial count, use the same sample that was used for the main assessment (i.e. the dilution that was used for the other organisms, typically 1:5).
  - a. Bacterial counts are often performed at higher dilutions than the main assessment because of the density of bacteria within a sample.
  - b. It is unnecessary to make a new sample for the bacterial counts, unless a significant period of time has passed from the time that the sample was prepared, e.g., more than 2 hours. This is because the biology in the test-tube may change over time and may be different from that in the soil or compost sample being tested.
2. Starting with the dilution that was used to perform the main assessment, e.g. 1:5, 1:10, 1:50, etc. Continue diluting the sample using the dilution factor equation until only 15-50 bacteria can be counted in a whole FoV,  $\frac{1}{2}$  FoV, or  $\frac{1}{4}$  FoV. *Reference Figure 11.*
  - a. Typically bacterial counts cannot be performed until the sample in the test tube has been highly diluted, almost making it appear clear.
  - b. Extremely bacteria-rich samples will require dilutions of over 1:1000.

- Once an appropriate dilution has been achieved, perform the bacterial counts in each of the 5 Readings. (*Reference Lecture 7.1.4*). Whatever portion (whole, half or quarter) of the FoV being observed, remember to record that portion and adjust the formulas in the microscope assessment spreadsheet, accordingly. If you decide to count the Bacteria in a  $\frac{1}{4}$  FoV, always count the same quadrant in every FoV, to eliminate bias.

When counting the “Whole Field” you will enter the total number of bacteria counted per Field observed. When using the “ $\frac{1}{2}$  Field” option to count bacteria, you will take your total and multiply by 2. If using the “ $\frac{1}{4}$  Field” option to count bacteria, then multiple the total counted by 4.

**Figure 11**



### 3.1.7 Calculation of the number of Fields of View on a Coverslip Area

**Key Point [43:41]** How to calculate the number of FoV per Coverslip Area

To be able to conduct biological assessments, the diameter of the FoV must be determined, along with the total number of FoV within the area of the coverslip used in the assessment. We are primarily interested in the diameter at a total magnification of 400X since it is at this magnification that we measure the length of all strand-like organisms (i.e. actinobacteria, fungi, and oomycetes) and the number of bacteria.

**Step 1:** First, determine the field number of your eyepiece. *Reference Lecture 2.1.4*

*Example: Eyepiece 10X/18. The field number is the diameter, in millimeters, of the circle of light observed when viewing through the eyepiece alone. In this case, the circle of light viewed through the eyepiece would be 18mm in diameter.*

**Step 2:** To calculate the diameter of light when viewed through the eyepiece and an objective lens, divide the field number by the magnification of the objective lens (*not total magnification*) and then convert to  $\mu\text{m}$ :

$$\text{FoV Diameter} = \text{Field Number} \div \text{Objective Magnification}$$

Calculated for an eyepiece with a field number of 18 and using the 40X objective:

$$\text{FoV Diameter} = 18 \text{ mm} \div 40\text{X} = 0.45\text{mm} = 450\mu\text{m}$$

(Alternatively you can use a stage micrometer to directly measure the FoV diameter.)

**Step 3:** Now we need to calculate the number of FoV per coverslip, which is dependent on the size of the coverslip and the diameter of the FoV.

To calculate the area of the cover slip: Example: 22 mm x 18 mm coverslip.

$$\text{Coverslip Area} = \text{Coverslip Length} \times \text{Coverslip Width}$$

$$22\text{mm} \times 18\text{mm} = 396 \text{ mm}^2$$

**Step 4:** Next, using the diameter of the FoV of 0.45mm and the formula for calculating the area of a circle, ( $\pi r^2$ ), where r is the radius (or half the diameter) and  $\pi$  is approximately 3.14:

$$\pi(0.5 * \text{diameter})^2 = \text{FoV Area}$$

$$3.14 * (0.5 * 0.45\text{mm})^2 = 0.159 \text{ mm}^2$$

**Step 5:** Finally, divide the area of the cover slip by the area of one field of view to obtain the number of FoV that are required to cover the full area of the cover slip. In our example:

$$\text{Coverslip Area} \div \text{FoV Area} = \text{Number of FoV per Coverslip}$$

$$396 \text{ mm}^2 \div 0.159 \text{ mm}^2 = 2491 \text{ Fields of View}$$

**Table 2** - Number of FoV per Coverslip Chart

Coverslip Size (mm)	Coverslip Area (mm <sup>2</sup> )	Field # on Eyepiece	FOV Diameter (mm) (calculated for the 40X objective is field #/objective)	Area of Field (mm <sup>2</sup> )	# of Fields at 400X
18 X 18	324	18	0.45	0.1590	2038
18 X 22	396	18	0.45	0.1590	2491
20 X 20	400	18	0.45	0.1590	2516
22 X 22	484	18	0.45	0.1590	3045
18 X 18	324	18.5	0.4625	0.1679	1930
18 X 22	396	18.5	0.4625	0.1679	2358
20 X 20	400	18.5	0.4625	0.1679	2382
22 X 22	484	18.5	0.4625	0.1679	2882
18 X 18	324	20	0.5	0.1963	1651
18 X 22	396	20	0.5	0.1963	2018
20 X 20	400	20	0.5	0.1963	2038
22 X 22	484	20	0.5	0.1963	2466
18 X 18	324	22	0.55	0.2375	1364
18 X 22	396	22	0.55	0.2375	1668
20 X 20	400	22	0.55	0.2375	1684
22 X 22	484	22	0.55	0.2375	2038
18 X 18	324	25	0.625	0.3066	1057
18 X 22	396	25	0.625	0.3066	1291
20 X 20	400	25	0.625	0.3066	1304
22 X 22	484	25	0.625	0.3066	1578

**3.2 GLOSSARY TERMS** (Please refer to the glossary on page 41)

The following glossary terms are used in this lecture: ***Bdellovibrio***; **Corkscrew**; **Corynebacterium**(singular) /**Corynebacteria**(plural); **Coryneform**; **Diplobacillus**(singular)/**Diplobacilli**(plural); **Diplococcus**(singular) /**Diplococci**(plural); **Encapsulated Diplococci**; **Eukaryote**; **Fusobacterium**; **Helical Form**; **Helicobacter pylori**; **Palisade**; **Pneumococcus**; **Prokaryote**; **Sarcina**; **Stalked Bacteria**; **Staphylococci**; **Streptobacillus**; **Taxonomy/Taxonomist**; **Tetrad**; **Undulate**.

# LECTURE 4

## Lecture 4: Bacterial Morphology (Part 2) [39:42]

### 4.0 OVERVIEW

Lecture 4 continues with the concept of assessing bacteria by morphology. This lecture explains the difference between the “Qualitative Approach” and the “Quantitative Approach” when doing biological assessments.

### 4.1 LECTURE CONTENTS

#### 4.1.1 Bacterial Biomass: A Qualitative Approach

The Qualitative Approach can be used when approximation of biomass or numbers of microorganisms is acceptable. When completing a biological assessment that requires accuracy, the Quantitative Approach should be used, which will give the most accurate results. With the Qualitative Approach a biological assessment can be completed more quickly, but this takes practice.

#### 4.1.2 Example of the proper dilution when counting bacteria using the Quantitative Approach

#### 4.1.3 Examples using microscope images to assess with the Qualitative Approach for bacterial biomass

### Key Point [7:13] - Standard Deviation

Standard Deviation is a means of assessing the variability of the data collected. For the purposes of this exercise you need to understand one thing i.e. that if the Standard Deviation is equal to, or greater than the mean, then the results are not sufficiently consistent to be of any value, and we treat the result as being equal to zero. So if the Standard deviation for your fungal count is greater than the mean, your fungal count should be treated as zero.

In practical application, if the standard deviation is equal to 50% of the mean, the mean is somewhat unreliable, but acceptable. The larger the standard deviation becomes, the less valid the data becomes. Ideally, we would like to see the standard deviation <20%.

Please see section 7.1.4 for additional guidelines on the use of Standard Deviation when evaluating the results of a microscope assessment.

Here are some resources for those who wish to understand the Standard Deviation better:

<https://www.mathsisfun.com/data/standard-deviation.html>

<https://www.mathsisfun.com/data/standard-deviation-formulas.html>

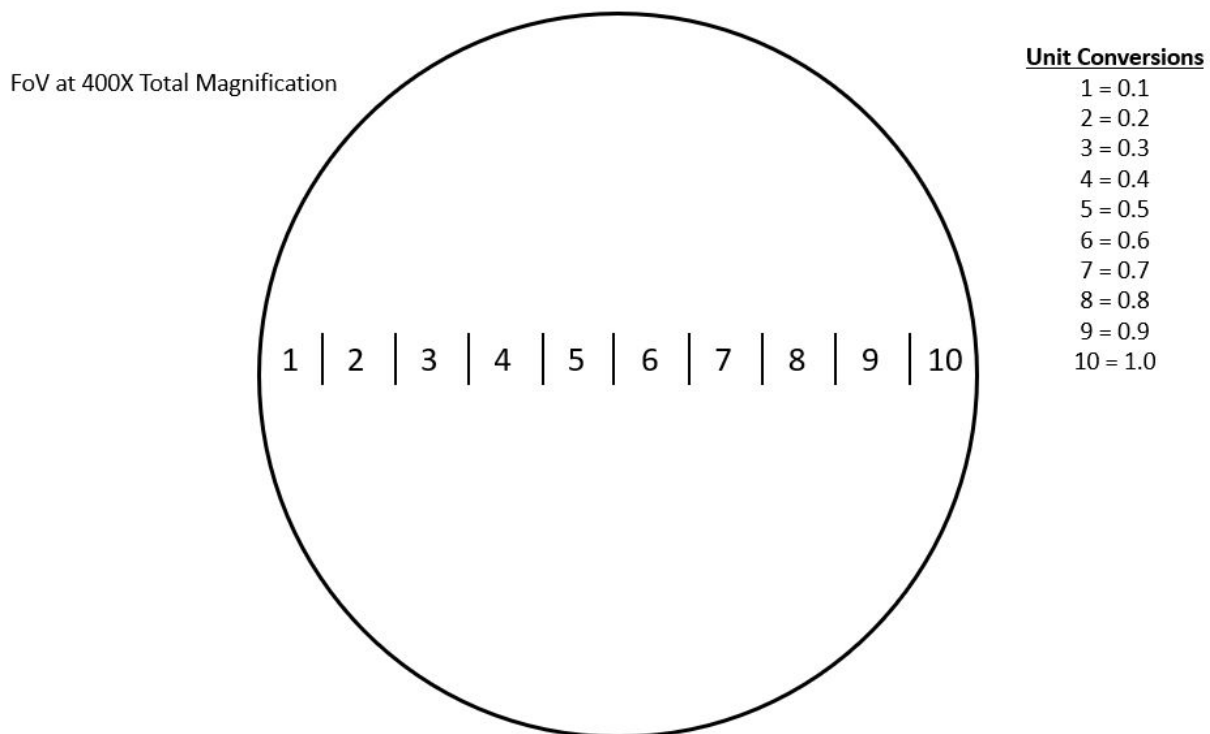
#### 4.1.4 Measuring Filamentous Microorganisms

**Key Point [19:11]** - How to measure filamentous microorganisms, e.g. actinobacteria, fungi, and oomycetes.

To measure a filamentous organism, start off by visually dividing the diameter of the FoV into 10 equal parts. Each of these parts will equal 0.1 units. These measurements must always be performed at 400X total magnification.

The next step is to estimate the length of the filamentous organism in units of FoV diameter. A fungal hypha that is equal to half the diameter of the FoV will have a length of 0.5 units. An actinobacterium that spans across the entire FoV will have a length of 1 unit.

**Figure 12**

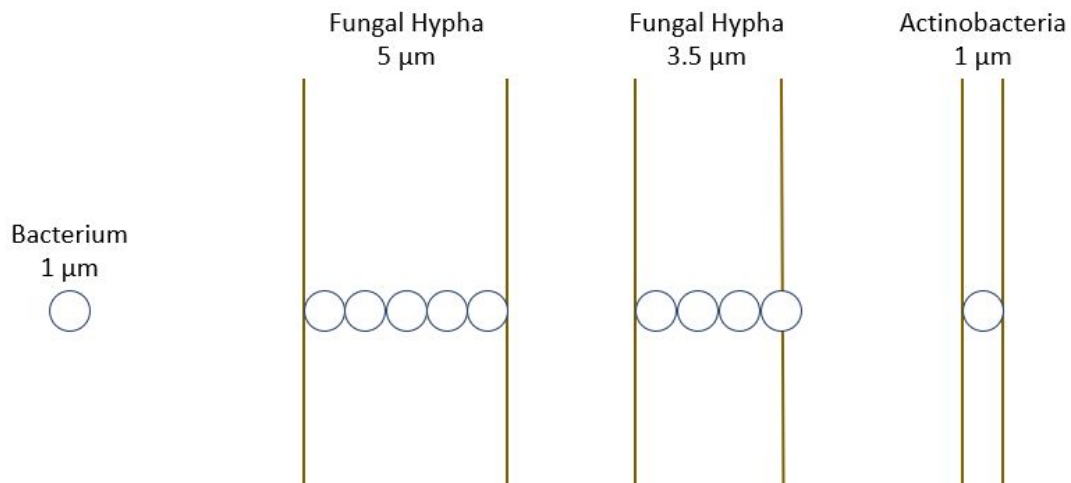


When measuring fungi, once the length has been measured, the next step is to estimate the diameter. To do this, find the smallest bacterium in the FoV that you are observing. The diameter of this bacterium will be 1 $\mu$ m. *Reference Figure 13.*

Now use that bacterium to visually estimate the width (or diameter) of the fungal hypha. This will enable you to estimate the diameter of the hypha in  $\mu$ m.

Note: In the microscope spreadsheet, the diameter for the actinobacteria is set at 1 $\mu$ m and it is not therefore necessary to estimate the diameter for this microorganism.

**Figure 13**



4.1.5 Electron Scanning Microscope image

4.1.6 Calculation for actinobacteria

## 4.2 GLOSSARY TERMS *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Fatty Acids; Gold/Palladium (Au/Pd); Standard Deviation.**

## 4.3 REFERENCES & FURTHER READING

"Microbe Library" in the FC Forum

<https://www.soilfoodweb.com/fc-forum>

# LECTURE 5

## Lecture 5: Fungal Morphology (Part 1) [28:36]

### 5.0 OVERVIEW

Lecture 5 is the beginning of a 3 part lecture series on fungi and other filamentous microorganisms. The basic morphological characteristics outlined in FC1 are reviewed and expanded upon. Differentiation between the “beneficial fungi” and the “disease-causing fungi” is covered in this lecture.

### 5.1 LECTURE CONTENTS

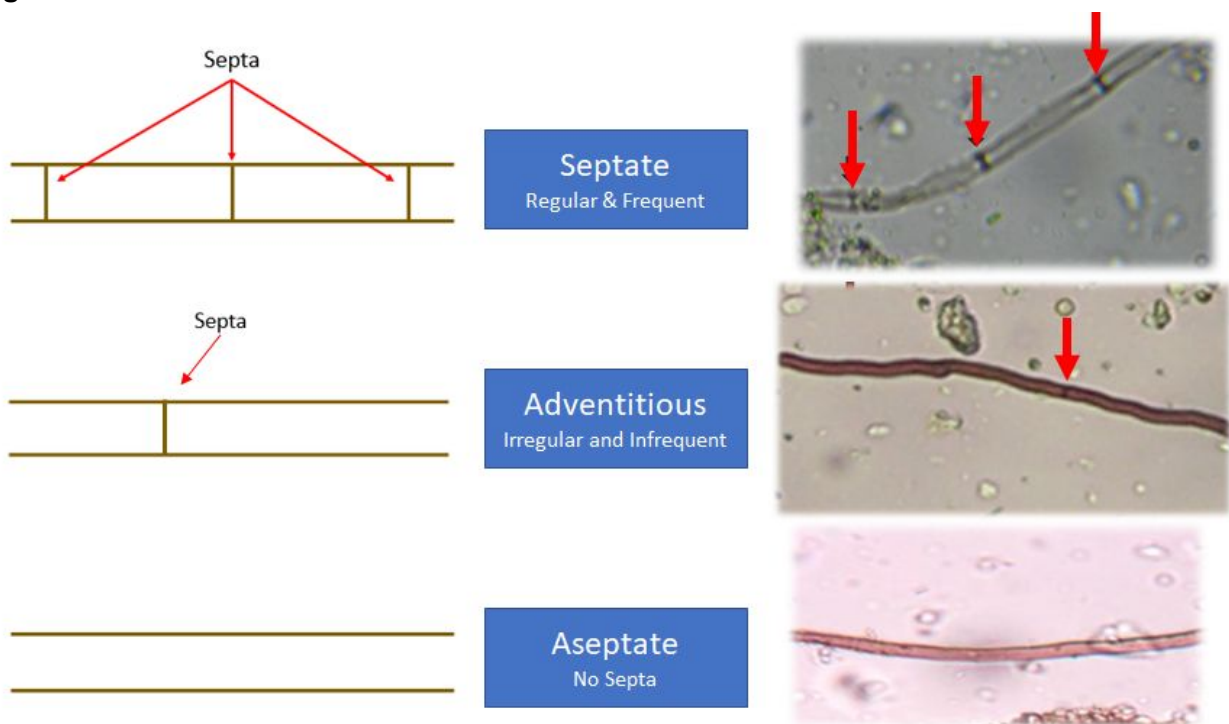
5.1.1 Fungal morphology: differentiation between beneficial and disease-causing fungi.

#### Filamentous Microorganisms

- (Actinobacteria 1 -1.5  $\mu\text{m}$ ) (*Not fungi, classified as bacteria*)
- Oomycetes 1.5 – 2.5  $\mu\text{m}$ , clear, colorless, aseptate or adventitiously septate
  - Zygomycetes (zoospores)
- Deuteromycetes 2 – 2.5  $\mu\text{m}$  (Fungi Imperfecti)
- Ascomycetes > 2  $\mu\text{m}$ , (septate)
- Basidiomycetes > 3  $\mu\text{m}$ , septate

**Key Point [4:41]** Identifying septate, adventitiously septated, and aseptate fungi

Figure 14



5.1.2 A graphic example of fungi broken down into Kingdom Mycota

5.1.3 Characteristics of fungi relevant to biological assessments

**Key Point [14:17]** Image of cellulose

**Figure 15**



Source: Susan Delhommer

5.1.4 Characteristics of Beneficial Fungi

**Hyphae:**

- **Color:** Colored in some way, compare the color of the hyphae to the sample background. The darker the better.
- **Diameter:** Beneficial fungi have diameters wider than 2.5 - 3 $\mu$ m. A few exceptions (Rhizoctonia). The diameter is uniform along the length of the hypha.
- **Cross-walls:** If present, uniform distance between septa indicate beneficial fungi. Clamp connections ALWAYS indicate beneficial fungi (Basidiomycetes).
- **Oxalic acid crystals:** Beneficial fungi make oxalate crystals to store charged nutrients.
- Fungi will not fray, instead they have clean, square breaks.

**Key Point [16:04]** Image of Rhizoctonia with indentation morphological characteristic

**Figure 16**

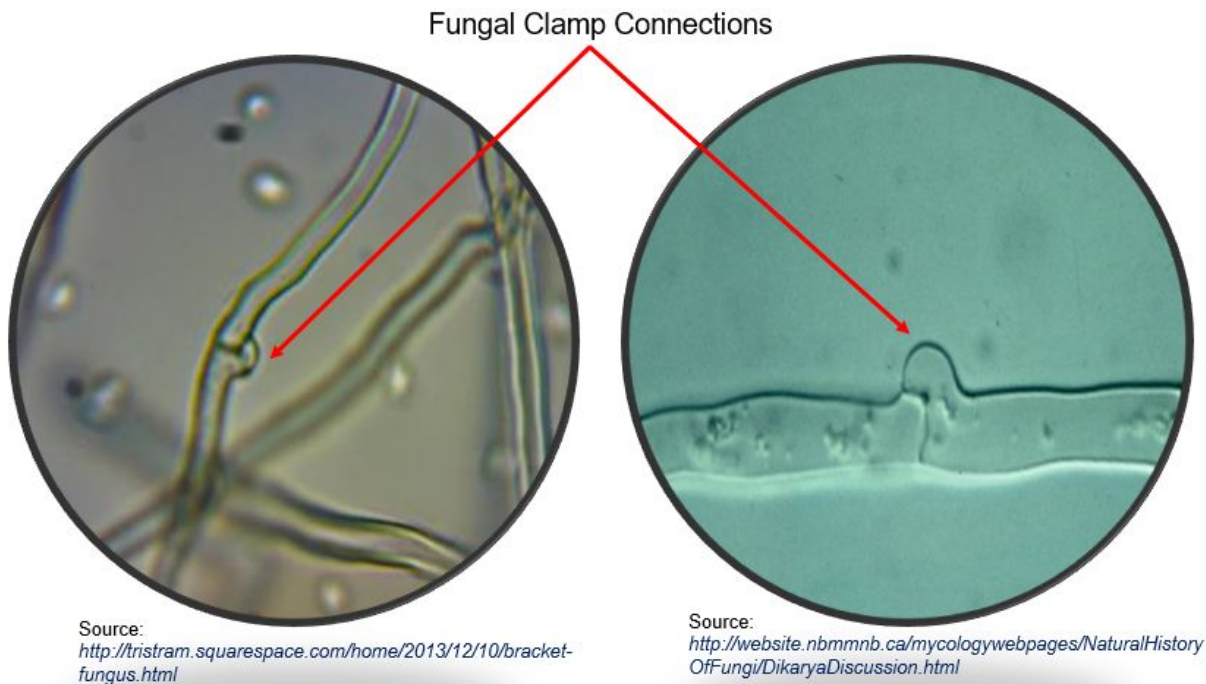
Indentation of Rhizoctonia hyphal branch; which is a characteristic of this genus.



Source: <https://tdl.wisc.edu/rhizoctonia-brown-patch/>

**Key Point [18:46]** Image of clamp connection on a fungal hypha

**Figure 17**

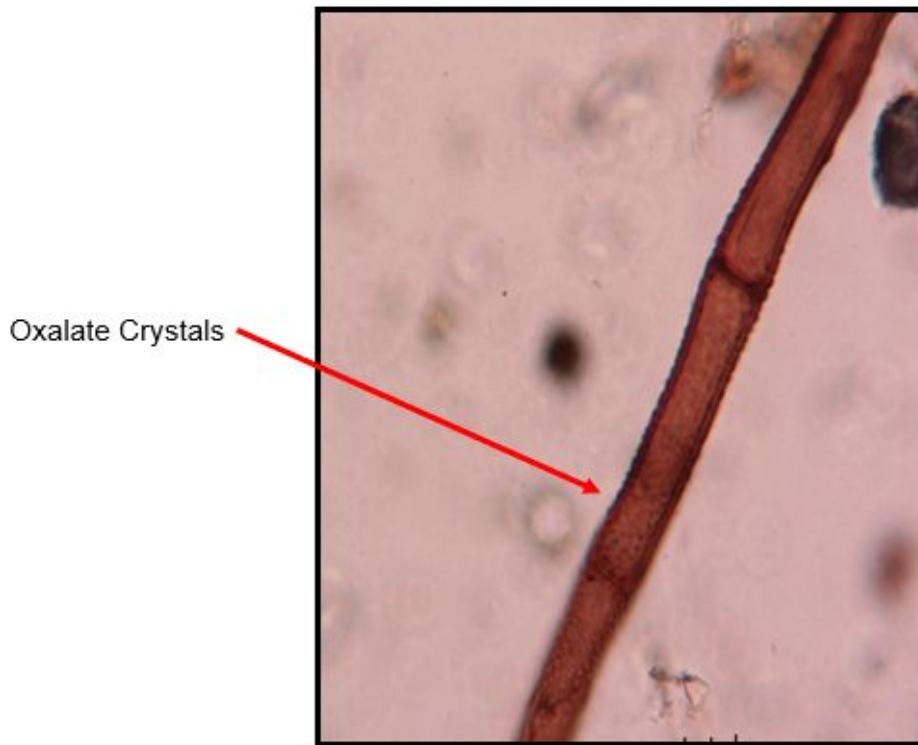


Source: <http://tristram.squarespace.com/home/2013/12/10/bracket-fungus.html>

Source: <http://website.nbmmnb.ca/mycologywebpages/NaturalHistoryOfFungi/DikaryaDiscussion.html>

**Key Point [20:18]** Image of oxalate crystals on a fungal hypha

**Figure 18**



#### 5.1.5 Characteristics of Disease-causing Fungi

##### Hyphae:

- **General Appearance:** More-or-less uniform diameter but the hypha may be lumpy, bumpy, with bubbly cytoplasm. Big clumps, or wefts, of hyphae.
- **Diameter:** Most disease-causing fungi have diameters narrower than 2 - 2.5 micrometers.
- **Color:** Clear, colorless; compared to background color.
- **Cross-walls:** Adventitious septa at best, usually no discernable cross-walls, no clamp connections.
- **Oxalic acid crystals:** None.
- **Spores:** Chlamydospores, sporangia, conidia common.

#### 5.2 GLOSSARY TERMS *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Actinomycete; Algal Fungi; Aseptate; Chlamydospores; Club Fungi; Conjugation Fungi; Deuteromycetes; Eumycota; Fungi Imperfecti; Indentation; Malaria; Oomycota; Phycomycetes; Sac Fungi; Secondary Invader; Sporangia; Zygomycetes.**

# LECTURE 6

## Lecture 6: Fungal Morphology (Part 2) [51:48]

### 6.0 OVERVIEW

Lecture 6 continues with part 2 of the Fungal Morphology lecture series. The series of images in this lecture covers a wide range of different microorganisms (with a special focus on fungi) and objects that will possibly be encountered when examining samples.

### 6.1 LECTURE CONTENTS

6.1.1 Examples of microscope images

### 6.2 GLOSSARY TERMS *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Bilayer**; ***Fusarium solani***; **Girdle**; **Mating Type**; **Reproductive Structure**; **Tripartite**; **Trypan Blue**.

# LECTURE 7

## Lecture 7: Fungal Morphology (Part 3) [54:57]

### 7.0 OVERVIEW

Lecture 7 continues the Fungal Morphology lecture series with further explanations of calculating fungal biomass for a biological assessment. How fungi grow while in a tea brewer is explained, along with greater detail of the symbiotic relationship that is formed between mycorrhizal fungi and plants.

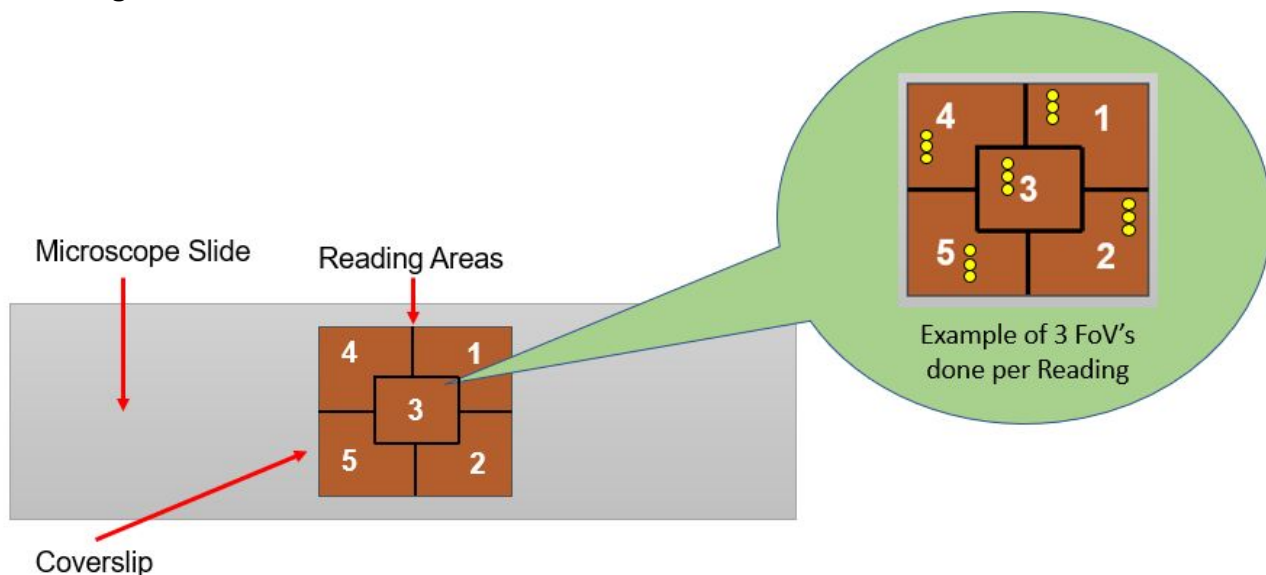
### 7.1 LECTURE CONTENTS

- 7.1.1 Why bacteria and fungi need to each be converted into biomass
- 7.1.2 Measuring fungal length and diameter - *Reference Lecture 4.1.4, Figure 11 & 12*
- 7.1.3 Converting fungal measurements first into biovolume then into biomass  
(This same equation is used for calculating oomycetes.)
- 7.1.4 Dividing a coverslip into Readings during the process of biological assessment

**Key Point [16:09]** How to section a coverslip into “Readings” and “Fields” and determining how many “Fields” per “Reading” to do in a biological assessment

When performing biological assessments, imagine dividing the coverslip into 5 sections. You will then take one Reading from each of these sections. A Reading will usually comprise several Fields of View.

**Figure 19**



## Determining the Number of Fields to Observe Per Reading

**Step 1:** Go to the first reading area and the first FoV. Count the following organisms and record the data: Actinobacteria, Fungi(beneficial and/or Oomycetes) & Protozoa.

**Step 2:** If all three groups of beneficial microorganisms are present then you can move onto the second reading area and set the number of FoV per reading area to 1. You can then complete the assessment by counting the organisms in one FoV in each of the remaining reading areas. Remember to visit all 5 reading areas.

If all three groups of beneficial microorganisms have not yet been observed and recorded, then move to the second FoV in the same reading area - this should be directly below (adjacent to) the first FoV.

**Step 3:** Now count the microorganisms in the second FoV (still in the first reading area) and record the data. If you have now observed microorganisms in each of the beneficial groups, you can complete the assessment by counting the organisms in two FoV in each of the remaining reading areas. Remember to visit all 5 reading areas.

**Step 4:** Continue to observe FoVs in reading area 1 until you have either:

- a) observed microorganisms in each of the beneficial groups, at which point you can set the number of FoVs per reading area, and then move onto reading area 2, or
- b) completed 10 FoVs in reading 1, without having observed microorganisms in each of the three beneficial groups. At this point you must conclude that the sample is deficient and terminate the assessment.

## Evaluating Data

Evaluate the mean and standard deviation for the various organism groups. If the standard deviations of most of your organism groups are less than 50% of the mean, this indicates that there is not excessive variability and that the data from this sample can be considered reliable.

If any single group has a Standard Deviation above 70 percent of the mean, then the results of that group are to be considered statistically unreliable.

If the standard deviation for Beneficial Fungi, Oomycetes and/or Actinobacteria \*\* is greater than 70 percent of the mean, go back and prepare a new slide with a new drop of your sample and start again (you may want to go all the way back to the beginning of the process and prepare a new dilution). If the standard deviation is still >70 percent of the mean, then you can conclude that there are not enough beneficial fungi in that sample to produce reliable data.

\*\* This does not apply to Bacteria and/or Protozoa because: 1. Bacterial SD tends to be very low. 2. Protozoan SD tends to be very large.

- 7.1.5 Converting from Biomass in a FoV to Biomass/g
- 7.1.6 Fungal growth in brewers explained
- 7.1.7 Images of fungi in teas and visual clues for the identification of different types of fungi encountered in compost, organic material and soil.
- 7.1.8 Mycorrhizal Fungi and their symbiotic relationship with plants

**Key Point [38:13]** The difference between a Compound Shadowing Microscope and an Epifluorescent Microscope

Throughout these courses, you have heard Dr. Ingham mention an Epifluorescent Microscope for assessing active microorganisms. This type of microscope is not necessary for completing the *basic* biological assessments of the Soil Food Web. This is a specialized tool used to assess the *active* count/biomass of microorganisms.

Active Biomass can be used to compare the metabolic activity between different groups of microorganisms. If active fungal biomass is greater than that of bacteria, then we can infer that the soil/compost is likely to become more fungal over the next period of time.

Epifluorescent microscopes can also be used to assess the level of foliar coverage by beneficial microorganisms, following a compost tea application.

The most commonly used chemical stain for this type of Soil Food Web assessment is fluorescein diacetate.

Epifluorescent microscopes can also be used to observe mycorrhizal colonization in root systems.

- 7.1.9 Images of mycorrhizal fungi

## 7.2 GLOSSARY TERMS *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Acidified; Chockablock; Fluorescein Diacetate; Interstitial; Rhizomorph; Sclerotium; Suberize; Symbiosis.**

### 7.3 REFERENCES & FURTHER READING

Ames, R.N., E.R. Ingham and C.P.P. Reid. (1982). Ultraviolet-induced auto fluorescence of arbuscular mycorrhizal root infections: An alternative to clearing and staining methods for assessing infections. *Can. Jr. Microbiol.* 28:351-355.

Ames, R.N., C.P.P. Reid and E.R. Ingham. (1984). Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. *New Phytol.* 96:555-563.

Ingham, E.R., J.A. Trofymow, R.N. Ames, H.W. Hunt, C.R. Morley, J.C. Moore and D.C. Coleman. (1986). Trophic interactions and nitrogen cycling in a semiarid grassland soil. Part I. Seasonal dynamics of the natural populations, their interactions and effects on nitrogen cycling. *J. Applied Ecology* 23:597-614.

Ingham, E.R., J.A. Trofymow, R.N. Ames, H.W. Hunt, C.R. Morley, J.C. Moore and D.C. Coleman. (1986). Trophic interactions and nitrogen cycling in a semiarid grassland soil. Part II. System responses to removal of different groups of soil microbes or fauna. *J. Applied Ecology* 23:615-630.

Hunt, H.W., D.C. Coleman, E.R. Ingham, R.E. Ingham, E.T. Elliott, J.C. Moore, C.P.P. Reid and C.R. Morley. (1987). The detrital food web in a short grass prairie. *Biol. Fert. Soil* 3:57-68.

Moore, J.C., E.R. Ingham and D.C. Coleman. (1987). Inter- and Intraspecific feeding selectivity of *Folsomia candida* (Willem) (Collembola, Isotomidae) on fungi: Method development and ecological consequences. *Biol. Fert. Soil* 5:6-12.

# LECTURE 8

## Lecture 8: Protozoan Morphology [53:27]

### 8.0 OVERVIEW

In this lecture, morphology, motility and other characteristics are covered in more detail. In order to more easily classify each microorganism during biological assessments.

This lecture also explains how to identify rotifers and parasites, along with what it means when these microorganisms are observed in a sample.

### 8.1 LECTURE CONTENTS

#### 8.1.1 Protozoan morphology comparison

**Table 3**

- **Flagellates - AEROBIC**
  - Round, pear, teardrop, banana
  - Rolling, bumbling motion, one or more flagella
  - Cysts – single layer outer membrane, small, round
- **Amoebae - AEROBIC**
  - Very slow oozing movement
  - Some species have a test (shell) around their bodies (Testate amoebae)
  - Cyst – double outer membrane that is often ornamented
- **Ciliates** – indicate **ANAEROBIC** conditions
  - Very fast, many cilia (short hairs) may cover the whole body or only part, usually larger than other protozoa
  - Cyst - single layer outer membrane, large, round

#### 8.1.2 Protozoan motility review

#### 8.1.3 Microscope images of protozoa

#### 8.1.4 Explanation on what rotifers and insect larva indicate when observed in a sample

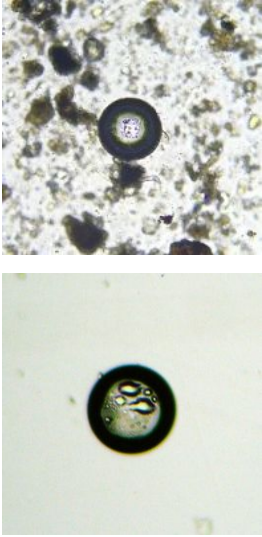
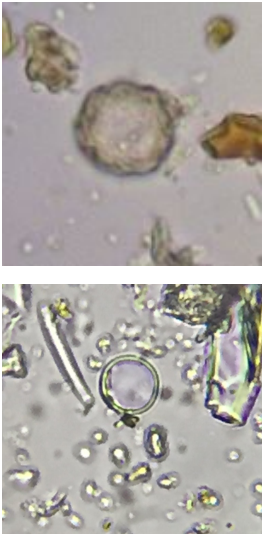

#### 8.1.5 Parasite morphology and what they indicate when observed in a sample

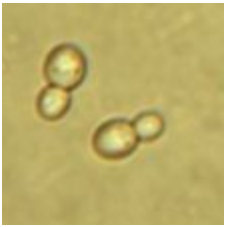


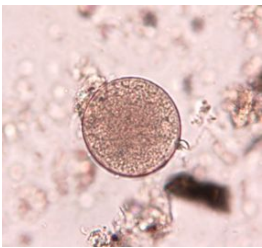
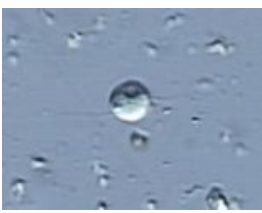
#### 8.1.6 Calculation for protozoa per gram or ml of sample

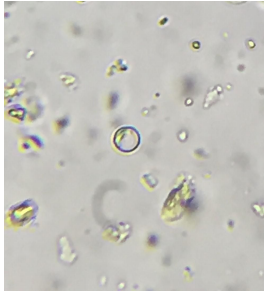
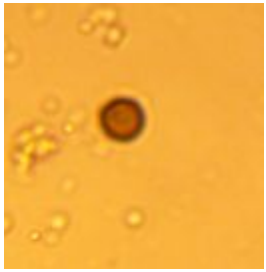
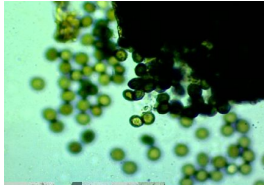
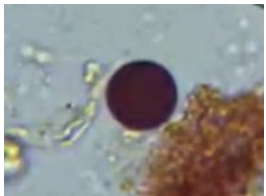

#### 8.1.7 Quick reference guide of different round organisms (or objects) that are found in biological assessments.

Please note that in the table below, images are not to scale and organisms may appear larger than when observed in a sample.

**Table 4**

Object	Size Range	Interior	Notes	Picture(s)
Air bubble forming around oil droplets	3µm +	Clear, or may have oil droplets inside	Walls change in apparent thickness as you focus the microscope up and down	
Amoeba cyst	<p>Typically about the same size as the active organism</p> <p>Typically, in soil, amoeba cysts can be as small as 3µm and as large as 15-20µm</p>	Clear	<p>Always double-walled (sometimes one wall is more distinct than the other)</p> <p>One wall may be ornamented</p>	
<p>Arcella</p> <p>(The only round genus of testate amoebae)</p>	10µm - 25µm	<p>Contains cytoplasm, bacteria and debris from outside environment</p> <p>Vacuoles form and disappear as waste products are released outside of the cell</p>	<p>Amoeba builds a test (shell) around itself</p> <p>Flattened, circular organism with a visible opening in the center</p> <p>Empty tests should not be counted in an assessment - must</p>	

			see amoeba inside	
Budding Yeast  (Yeasts are an anaerobic form of fungi)	4µm - 25µm	Can be clear, golden, yellow, possibly red  Viable cells will contain cytoplasm and vacuoles. (Non-viable cells will be empty/clear/colorless)	Generally not perfectly round; tend to be oblong or pear-shaped  Typically always form a cluster. Within a cluster, there will almost always be some yeasts budding (smaller cell growing out from the mother cell)	
Cercozoa	5µm - 10µm  Approximately the same size as a larger flagellate cyst, but typically not as large as ciliate cysts	Movement and activity (cytoplasm, bacteria and vacuoles) inside the cell differentiates it from spores and cysts	Pseudopods (very slow moving/oozing structures) ooze out 1µm from the amoeba  Cytoplasm remains inside of the cell wall if the coverslip is tapped	
Ciliate cyst	Typically about the same size as the active organism  Typically up to 5-10 times larger than most flagellate cysts	Clear	Always single-walled	
Ciliate entering into dormant form (cyst)	Typically about the same size as the active organism  Typically up to 5-10 times larger than most flagellates	Even, grainy material  Interior can contain bacteria and vacuoles	Always single-walled	
Flagellate	"micro" flagellates (3µm - 5µm) can be difficult to distinguish from bacteria  Typical flagellates are 5µm - 15µm	In typically-sized flagellates, bacteria, cytoplasm and vacuoles are visible moving around inside the cell	Looking for one or more flagella (can be on the same side of the body or opposite sides)  Can move as fast as a ciliate, although rare to observe	

Flagellate cyst	Typically about the same size as the active organism (5µm - 15µm)	Clear	Always single-walled	
Fungal spore (Dormant stages of fungi)	2.5µm up to 10µm	Solid color throughout  Can be brown, dark brown, tan or colorless	Most round fungal spores are asexually produced  Sexually-produced spores are round/oval shaped in little sacs with 8-12 spores inside it (ascus)	 
VAM Spore (Endomycorrhizal fungal spores)	Generally, double the size of a typical fungal spore (5µm - 20µm)	Always dark brown in color  Often have inclusions (deposit of phosphate) inside the spore. These appear as darker patches inside the spore	Generally they will be very round	 

**8.2 GLOSSARY TERMS** *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Colpodea**; **Conidium**(singular)/**Conidia**(plural); **Fission**; **Pedipalps**; **Sessile**; **Test**; **Vorticella**.

# LECTURE 9

## Lecture 9: Nematode Morphology [1:10:31]

### 9.0 OVERVIEW

Lecture 9 reviews the previous knowledge learned from FC1, expounding on nematode functional groups and identifying their morphological characteristics within these functional groups. Using the soil food web method only requires that nematodes are classified into functional groups, not by genus or species.

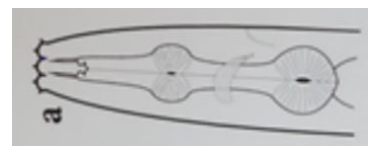

### 9.1 LECTURE CONTENTS


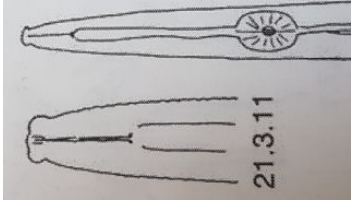

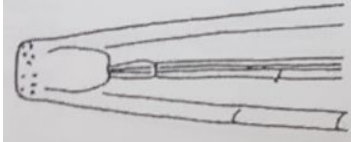
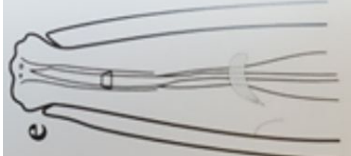
9.1.1 Nematodes explained and classified by functional groups

9.1.2 Nematode morphological characteristics

#### Key Point [12:18]

Table 5

Type (Functional Group)	Size	Bulbs	Shape of Mouth	Sketch
<b>Bacterial Feeders</b>	First larval stage: very small  Adults: medium	1 or 2  (Can have only terminal bulbs)	- Simple mouth: cylindrical /rectangular/V-shaped or no apparent mouth  - Fancy/ornate lips with or without whiskers outside the mouth	
<b>Fungal Feeders</b>	Juvenile stage: small  Adults: medium	0	- Features a spear but no knob  - The esophagus will be narrow, and can have a median swelling at a certain point before going into the intestines	

<p><b>Root Feeders</b></p>	<p>Larval stage: very small</p> <p>Adults: medium</p>	<p>1 (median bulb)</p>	<p>- Features a spear AND <u>large</u> knobs (2) at the end of the spear</p> <p>- Esophagus will have an additional swell partway down before going into the intestines.</p>	
<p><b>Switcher</b></p> <p>- will usually eat fungi but may switch to plant roots if fungi unavailable.</p>	<p>Larval stage: small</p> <p>Adults: medium</p>	<p>1 (typically a median)</p>	<p>- Features a spear AND <u>small</u> knobs</p> <p>- Esophagus will have an additional swell partway down before going into the intestines</p>	
<p><b>Predatory</b></p> <p>- eat other nematodes</p>	<p>Larval stage: medium</p> <p>Adults: Very large</p>	<p>0</p>	<p>- Features a large mouth and a tooth, used to pull in its prey</p>	
<p><b>Bacterial Omnivore</b></p> <p>- will eat bacteria, cysts, spores, algae, protozoa</p>	<p>Larval stage: medium</p> <p>Adults: large</p>	<p>0</p>	<p>- Wide mouthed, not as deep a mouth as the predatory nematode</p> <p>- Can have a number of denticles (tiny teeth)</p> <p>- May feature some setae (small hairs) on or around the lips</p>	
<p><b>Fungal Omnivore</b></p> <p>- will typically eat fungi, but may also ingest cyst, spores</p>	<p>Larval stage: medium</p> <p>Adults: large</p>	<p>0</p>	<p>- Large spear</p> <p>- No knob/stylus</p> <p>- Esophagus will swell partway down before going into the intestines</p>	

9.1.3 Examples of microscope images of nematode morphological characteristics for each functional group

#### 9.1.4 Calculations for Nematodes per gram or ml of sample

### 9.2 GLOSSARY TERMS *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Alga/Algae; Basal Knobs; Criconemoides; Dorylaimellus; Exoskeleton; Meloidogyne; Pratylenchus; Root-knot Gall/Root Gall; Wilsonema.**

# LECTURE 10

## Lecture 10: Microarthropods, Algae, & Pollen [24:17]

### 10.0 OVERVIEW

Lecture 10 covers the general morphological characteristics of microarthropods, algae, and pollen..

### 10.1 LECTURE CONTENTS

10.1.1 General morphological characteristics of microarthropods

10.1.2 General morphological characteristics of algae

10.1.3 General morphological characteristics of pollen

### 10.2 GLOSSARY TERMS *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Algal Bloom; Antenna(singular)/Antennae(plural); Centipede; Collembola; Filamentous Algae; Millipede; Photosynthetic Pigment; Pollen; Springtails; Symphyla/Symphylan; Water Bear.**

### 10.3 REFERENCES & FURTHER READING

The Microbehunter Microscopy - aka *"The MicrobeHunter Blog"*  
<http://www.microbehunter.com/microbehunter-blog/>

# LECTURE 11

**Lecture 11: Organic Matter, Mineral Material, etc. [35:57]**

## **11.0 OVERVIEW**

Lecture 11 covers miscellaneous microscope images of objects or materials that are occasionally encountered when conducting biological assessments. Although these objects/materials are not counted as any of the soil food web members, it is helpful to be familiar with them when you come across them in a sample.

## **11.1 LECTURE CONTENTS**

11.1.1 Miscellaneous microscope images of (mostly) other types of materials that are not living organisms that can sometimes be seen when conducting biological assessments

## **11.2 GLOSSARY TERMS** *(Please refer to the glossary on page 41)*

The following glossary terms are used in this lecture: **Germ; Stilbene; Ventral/Ventrally; Xylem.**

# COURSE GLOSSARY

**Aberrant** - (*Biology*) diverging from the normal type.

**Acidified** - to make or become acidic, i.e., 1. increase the number of free hydrogen ions, or 2. reduce the pH value below pH 7.0.

**Actinomycete** - this is an outdated name for Actinobacteria. These bacteria were formerly classified as fungi due to the filamentous character of the strands. However, the narrow diameter of the filaments, differences in nucleic acid base composition from fungi, differences in cell wall structures, etc., brought about the realization that these organisms are bacteria, not fungi, and should properly be called actinobacteria.

**Alga/Algae** - a large group of simple, nonflowering, typically aquatic plants, including seaweeds and single-celled forms. Algae photosynthesize using a number of different photosynthetic compounds such as chlorophyll, but they lack true stems, roots, leaves, and vascular tissue.

**Algal Bloom** - a rapid increase or accumulation in the population of algae in freshwater or marine systems; recognized by the discoloration in the water due to algal pigments.

**Algal Fungi** - any of various fungi that resemble algae from the classification of Phycomycete.

**Antenna(singular)/Antennae(plural)** - (*Zoology*) a pair of long, thin sensory appendages on the heads of insects, crustaceans, and some other arthropods.

**Aseptate** - (*Biology*) biology not divided into cells or sections by septa.

**Assess/Assessing** - evaluate or estimate the nature, ability, or quality of something, i.e. quantify biomass or numbers of microorganisms per gram or ml of a sample.

**Basal Knobs** - (*Nematology*) a prominent bump, swelling, bulge, or projection at the base of a spear or stylet of a nematode.

**Bdellovibrio** - a genus of Gram-negative, obligate aerobic bacteria. One of the more notable characteristics of this genus is that members can parasitize other Gram-negative bacteria and feed on biopolymers, e.g., proteins and nucleic acids, of their prey.

**Bilayer** - (*Biochemistry*) a film two molecules thick (formed, e.g., by lipids), in which each molecule is arranged with its hydrophobic end directed inward toward the opposite side of the film and its hydrophilic end directed outward.

**Centipede** - a predatory myriapod invertebrate with a flattened elongated body composed of many segments; most segments bear a single pair of legs.

**Cercozoa** - a group of single-celled eukaryotes recently classified into the protozoan family.

**Chlamydozoospores** - (in certain fungi) a thick-walled hyphal cell that functions as a spore.

**Chockablock** - (*British*) crammed full of people or things.

**Club Fungi** - any of various basidiomycetes (family Clavariaceae) with a simple or branched often club-shaped sporophore.

**Collembola** - (*Entomology*) an order of insects that comprises springtails.

**Colpodea** - a class of ciliates with a distinct morphology that is reniform (kidney-shaped) and are strongly convex on one side and concave on the other; about 200 species are common in freshwater and soil habitats.

**Compound Microscope** - a microscope consisting of an objective and an eyepiece mounted in a drawtube, an instrument for magnifying small objects, consisting of a lens of short focal length for forming an image that is further magnified by a second lens of longer focal length.

**Conidium(singular)/Conidia(plural)** - (*Botany*) asexual spores produced by various fungi at the tip of the specialized hyphae called a conidiophore.

**Conjugation Fungi** - species of fungi that use fusion of usually similar gametes with ultimate union of their nuclei for sexual reproduction.

**Corkscrew** - move or twist in a spiral motion.

**Corynebacterium(singular)/Corynebacteria(plural)** - a group of bacteria, a few species of which cause disease in humans and other animals, including diphtheria. Most species of this group do not cause disease.

**Coryneform** - a genus of bacteria bacilli (rod-shaped) that are Gram-positive and aerobic, generally these bacteria line up side-to-side resembling a picket fence in appearance.

**Criconemoides** - a soil-dwelling, root-feeding nematode genus of the family Criconematidae having cuticle divided into annuli that give an effect of the appearance of a Japanese pagoda (pseudosegmentation).

**Diffuse/Diffused** - when the concentration of a chemical in one place is higher than another, whereupon the chemical will diffuse into the place of lower concentration, such that both areas will ultimately be at equal concentrations.

**Deuteromycetes** - any fungi of the class Fungi Imperfecti that reproduce only asexually by means of spores (conidia or oidia) or by budding.

**Diffraction/Diffracted** - when light passes from one substance to another, light will be scattered, reducing the amount of resolution when using a microscope.

**Diplobacillus(singular)/Diplobacilli(plural)** - two bacilli linked end to end in a chain.

**Diplococcus(singular)/Diplococci(plural)** - two cocci linked together in a chain.

**Encapsulated Diplococci** - a pair of cocci that are completely covered by an exterior cell membrane; typically bacteria that causes tuberculosis.

**Dorylaimellus** - a genus of fungal-feeding nematode.

**Eukaryote** - (*Biology*) an organism consisting of a cell or cells in which the genetic material is DNA in the form of chromosomes contained within a distinct nucleus. A specific set of DNA bases occur in eukaryotes, as well as unique cell wall structures.

**Eumycota** - (*Biology*) a phylum of true fungi, distinguished from the fungus like slime molds, e.g., Myxomycota, and similar organisms, by having a mycelial thallus. In some classification systems, it is the class of Eumycetes.

**Exoskeleton** - (*Zoology*) a rigid external covering for the body in some invertebrate animals, especially arthropods, providing both support and protection.

**Extramatrix Spores** - a fungal spore that is developed outside the fungal hyphae.

**Fatty Acids** - carboxylic acid consisting of a hydrocarbon chain and a terminal carboxyl group, especially any of those occurring as esters in fats and oils.

**Filamentous Algae** - a type of algae often called "moss" or "pond scum," are characterized by long, hair-like strands, or filaments, of connected cells (often reaching two metres in length).

**Fission** - the action of dividing or splitting something into two or more parts.

**Fluorescein Diacetate** - a yellow crystalline dye (C<sub>20</sub>H<sub>12</sub>O<sub>5</sub>) with a bright yellow-green fluorescence in alkaline solution.

**Fungi Imperfecti** - a heterogenous phylum of fungi which lack a sexual phase, or of which the sexual phase is not known.

**Fusarium solani** - a species of filamentous fungi in the division Ascomycota, family Nectriaceae. It is a common soil fungus and colonist of plant materials. It was believed to be only beneficial until enough of the food web was decimated, at which point *Fusarium* will wipe out plants, overnight.

**Fusobacterium** - a genus of anaerobic, Gram-negative, non-spore forming bacteria that is widely known and studied as a human and animal pathogen.

**Germ** - is the reproductive part of a seed that germinates to grow into a plant; it is the embryo of the seed.

**Girdle** - something that encircles or confines.

**Gold/Palladium (Au/Pd)** - alloys of gold and palladium, are applied as conductive coatings to biological specimens and other non-conducting materials, such as plastics and glass, to be viewed in a scanning electron microscope.

**Helical Form** - of or shaped like a helix; spiral.

**Helicobacter pylori** - previously known as *Campylobacter pylori*, is a Gram-negative, microaerophilic bacterium usually found in the stomach/digestive tract and has a tendency to attack the stomach lining causing ulcers.

**Indentation** - a deep recess or notch on the edge or surface of something.

**Interpupillary Distance (IPD)** - the distance between the center of the pupils of two eyes.

**Interstitial** - (*Ecology*) the spaces between any solid material in the soil or aquatic sediments.

**Köhler Illumination** - a method of specimen illumination used for transmitted and reflected light (trans-and epi-illuminated) optical microscopy.

**Malaria** - a human disease that is caused by protozoan parasite (genus *Plasmodium*) in the red blood cells. It is transmitted by the bite of anopheline mosquitoes and causes periodic attacks of chills and fever.

**Mating Type** - the molecular mechanisms that regulate compatibility in sexually reproducing eukaryotes.

**Meloidogyne** - an endoparasitic nematode characterised by the female entering the root and causing 5 root cells to swell up to very large proportions thus giving the name of root-knot nematodes to this genus.

**Millipede** - a myriapod invertebrate with an elongated body composed of many segments, most of which bear two pairs of legs. Most kinds are herbivorous, shun light, and live in the soil or under stones and logs.

**Oomycota** - also known as oomycetes currently classified as a phylum of fungus like organisms in the kingdom Chromista. May occur as saprotrophs (living on decayed matter) or as parasites living on higher plants and can be aquatic, amphibious, or terrestrial; commonly called water molds. These fungal like organisms are unique because they have motile asexual spores, called zoospores, that look a lot like flagellates.

**Palisade** - (*Bacteria*) a particular arrangement called a palisade is produced by these bacteria which resembles a picket fence or Chinese letters.

**Pedipalps** - structures on microarthropods that lie on each side of the mouth and often perform a specialized function such as grasping or feeling.

**Photosynthetic Pigment** - the pigments responsible for the capture of light energy during the light-dependent reactions of photosynthesis, e.g. chlorophyll.

**Phycomycetes** - any of a large class (Phycomycetes) of lower fungi that are in many respects similar to algae and are now often assigned to subdivisions (such as Mastigomycotina and Zygomycota). Any of the lower fungi, which typically form a nonseptate mycelium.

***Pneumococcus*** - a strictly anaerobic bacterium associated with pneumonia and some forms of meningitis.

**Pollen** - a fine powdery substance, typically yellow, consisting of microscopic grains discharged from the male part of a flower or from a male cone. Each grain contains a male gamete that can fertilize the female ovule, to which pollen is transported by the wind, insects, or other animals.

***Pratylenchus*** - a genus of nematodes known commonly as lesion nematodes. They are parasitic on plants and are responsible for root lesion disease on many taxa of host plants in temperate regions around the world.

**Prokaryote** - (*Biology*) a microscopic single-celled organism that has neither a distinct nucleus with a membrane nor other specialized organelles. They have very specific types of DNA bases and unique cell wall structure occur in these organisms.

**Reproductive Structure** - (*Botany*) the structures of a plant involved in its reproduction.

**Resolution** - the process or capability of making distinguishable the individual parts of an object. The amount of light reaching the eye from the sample is critically important.

**Rhizomorph** - (*Botany*) an aggregation of fungal threads intertwining like the strands of a rope that frequently resembles a root and is characteristic of many basidiomycetes.

**Root-knot Gall/Root Gall** - an abnormal enlargement or swelling of or on the root of a plant commonly due to a parasitic organism, i.e. Meloidogyne.

***Rotylenchus*** - a genus of plant-parasitic nematodes (family Tylenchidae) that attack plant roots and underground stems.

**Sac Fungi** - any of various Ascomycetous fungi in which the spores are formed in a sac or ascus fungus.

***Sarcina*** - a genus of Gram-positive cocci bacteria in the family Clostridiaceae. The genus takes its name from the Latin word "sarcina," meaning pack or bundle, after the cuboidal cell associations they form during division along three planes.

**Sclerotium** - (*Botany*) the hard dark resting body of certain fungi, consisting of a mass of hyphal threads, capable of remaining dormant for long periods.

**Secondary Invader** - any species that is unable to successfully invade due to some inhibiting property of the recipient ecosystem. A secondary invader only becomes so after primary invaders, through their presence and influence, alter that inhibiting property and invasion can proceed.

**Sessile** - the organism stays in place and can not move about.

**Shadowing** - when an organism casts a shadow it increases contrast between the organism and the shadow such that the organism is easier to see.

**Sporangia** - a cell or structure in which spores are produced.

**Springtails** - common name for Collembola.

**Stalked Bacteria** - species of bacteria that have an appendix from their bodies that resembles a narrow stalk.

**Standard Deviation** - is a measure that is used to quantify the amount of variation or dispersion of a set of data values. (Also represented by the Greek letter sigma  $\sigma$  or the Latin letter s)

***Staphylococci*** - any of a genus (*Staphylococcus*) of nonmotile gram-positive spherical bacteria that occur singly, in pairs or tetrads, or in irregular clusters (a bunch of grapes) and include causative agents of various diseases (such as skin infections, food poisoning, and endocarditis).

**Stilbene** - (*Chemistry*) a colorless to slightly yellow, crystalline, water-insoluble solid, C<sub>14</sub>H<sub>12</sub>, used chiefly in the manufacture of dyes (stilbene dyes).

**Streptobacillus** - any of a genus (*Streptobacillus*) of nonmotile gram-negative rod-shaped bacteria in which the individual cells are often joined in a chain.

**Suberize** - as roots grow older complex compounds are stored in those roots making the roots difficult to decompose.

**Symbiosis** - an interaction between two different organisms living in close physical association; which may be positive, negative, or neutral. A mutually beneficial relationship between organisms would be called a mutualism a type of symbiosis.

**Symphyla/Symphylan** - (*Zoology*) a small class of myriapod invertebrates which resemble the centipedes. They are small eyeless animals with one pair of legs per segment, typically living in soil and leaf mold.

**Taxonomy/Taxonomist** - (*Biology*) the branch of science concerned with classification, especially of organisms.

**Test** - (*Zoology*) an external hard or firm covering (such as a shell) of many invertebrates (such as an amoeba or mollusk).

**Tetrad** - a group or set of four.

**Tripartite** - consisting of three parts.

**Trypan Blue** - a diazo dye used as a biological stain due to its absorption by fungal hyphae and not root systems.

**Undulate** - to move with a smooth up-and-down motion.

**Ventral/Ventrally** - (*Zoology/Botany*) of, on, or relating to the underside of an animal or plant; abdominal.

**Vorticella** - (*Zoology*) a single-celled aquatic animal with a contractile stalk and a bell-shaped body bearing a ring of cilia. It can move long distances by retracting the stalk, pushing off from the surface, sending the organism zooming through the soil solution to another place where it sets up house keeping.

**Water Bear** - the common name for a tardigrade. A minute invertebrates with a short plump body and four pairs of stubby legs, living in water or in the film of water on plants such as mosses.

**Wilsonema** - a genus of bacterial-feeding nematodes with delicate, ornate lip structures.

**Xylem** - (*Botany*) the vascular tissue in plants that conducts water and dissolved nutrients upward from the root and also helps to form the woody element in the stem.

**Zygomycetes** - a subclass of fungi of the class Phycomycetes characterized by gametangia that are morphologically alike and by sexually produced zygospores.

# REFERENCES FURTHER READING & LINKS

The Microbehunter Microscopy - aka "*The MicrobeHunter Blog*"

<http://www.microbehunter.com/microbehunter-blog/>