# MICROSCOPY WORKSHOP

M. Isabel Dominguez_BMC_September 2025

**OBJECTIVES: LEARN TO:**

**1.** Use compound and stereo microscopes at various magnifications.
**2.** Identify fungal structures including hyphae, reproductive structures and spores
**3.** Prepare fungal specimens for microscopic examination including staining techniques and slide mounting.

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## MICROSCOPES

### Stereo Microscope (Dissecting Microscope)

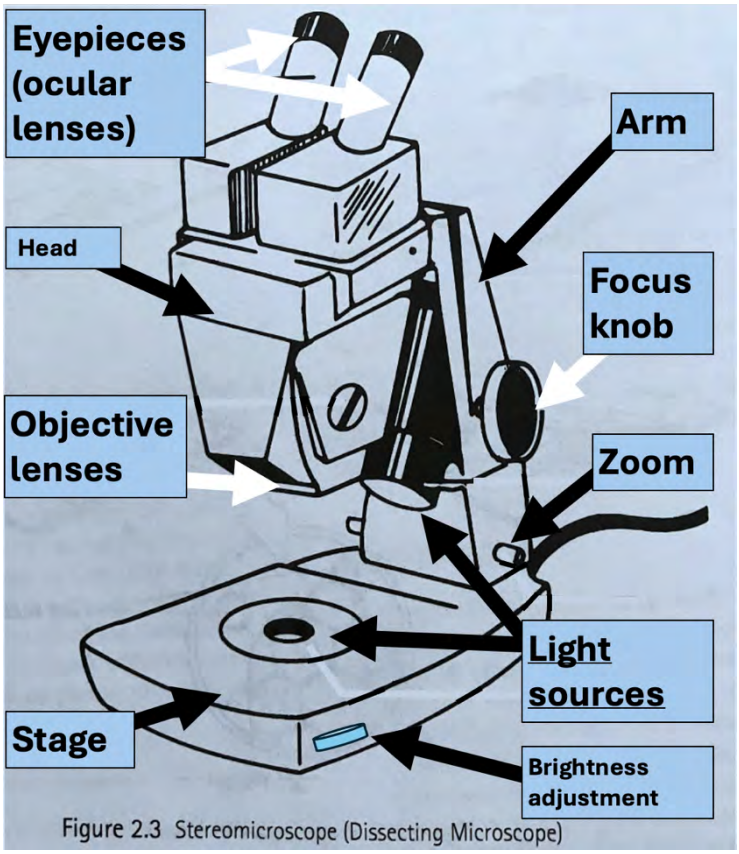


Figure 2.3 Stereomicroscope (Dissecting Microscope)

### Compound Light Microscope

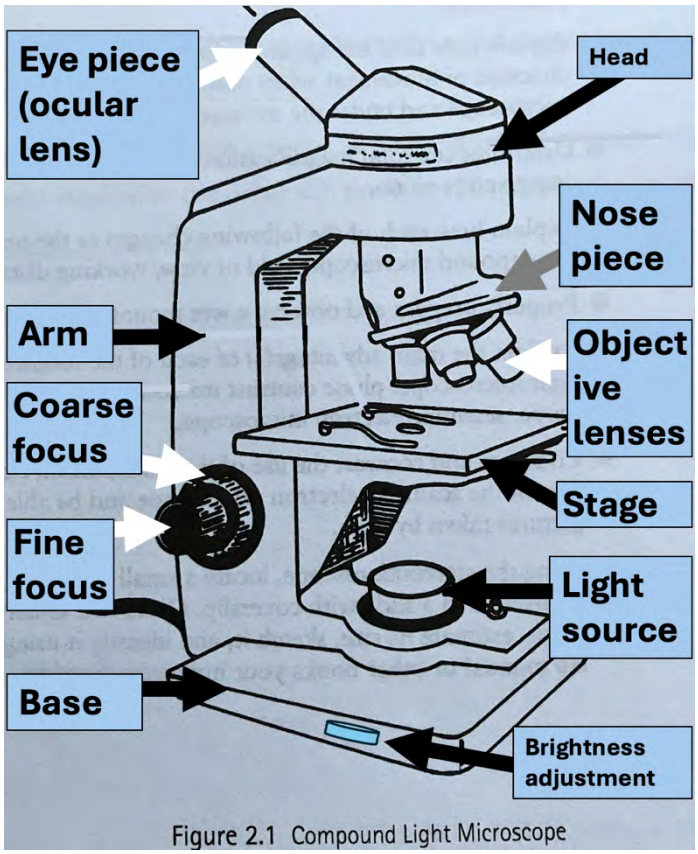


Figure 2.1 Compound Light Microscope

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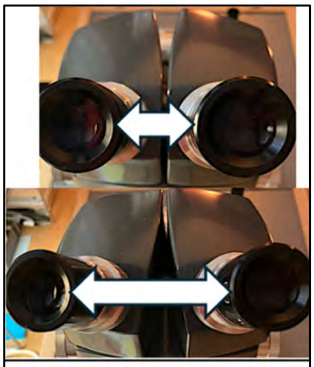
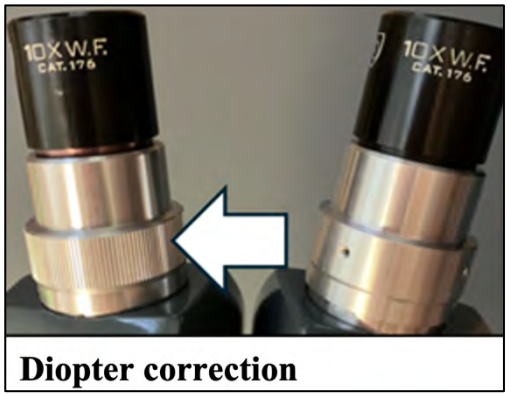
## EYEPIECES (Ocular Lenses):


- Name: Close to the eye
- Lens: magnifies the image. Fits inside the eyetubes (eyetubes may have different internal diameters) which are located in the HEAD
- Monocular microscope: one eyepiece
- Binocular microscope: two eyepieces
- Lens characteristics: - look at the numbers and letters written in the lens
  - **Magnification**: the power of the eyepiece; typically, 10X or 15X magnification
  - **Field of view**:
    - W.F. = Wide Field: wide field of view
    - Field number: the diameter that can be seen thought the eyepiece (e.g., 22 mm)
      – Note: the final field of view depends also on the objective lens used

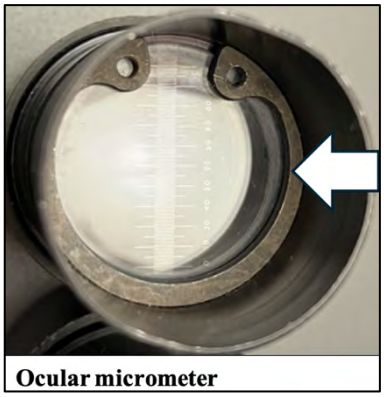
- Personal adjustments:
  - **Interpupillary distance adjustment**: hold with both hands and carefully move eyetubes until you see one single circular image (otherwise your eyes will be tired)
  - **Diopter correction**: knob on the left ocular lens used to correct for minor differences in vision between the eyes and get a clear image. *Note from David: get a clear image with the right lens first and then use the diopter to adjust the left lens*


Interpupillary distance adjustment



Diopter correction

- Cleaning eyepieces: use only lens paper. No chemicals, not cloths,…
- Pointer: projects a line into the eyepiece. Used to indicate an area in the specimen
- Ocular micrometer: small glass disc containing an arbitrary ruler scale inserted in one eyepiece. Needs to be calibrated for each magnification.


Ocular micrometer

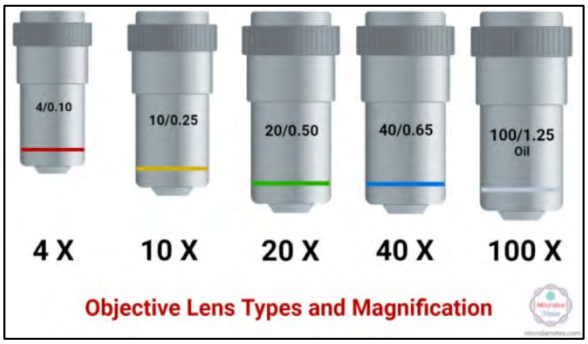
**OBJECTIVE LENSES:**

- Name: Close to the object (specimen)
- Lens: gather and magnify the image of the specimen. Mounted in a revolving turret or nosepiece in compound microscopes.

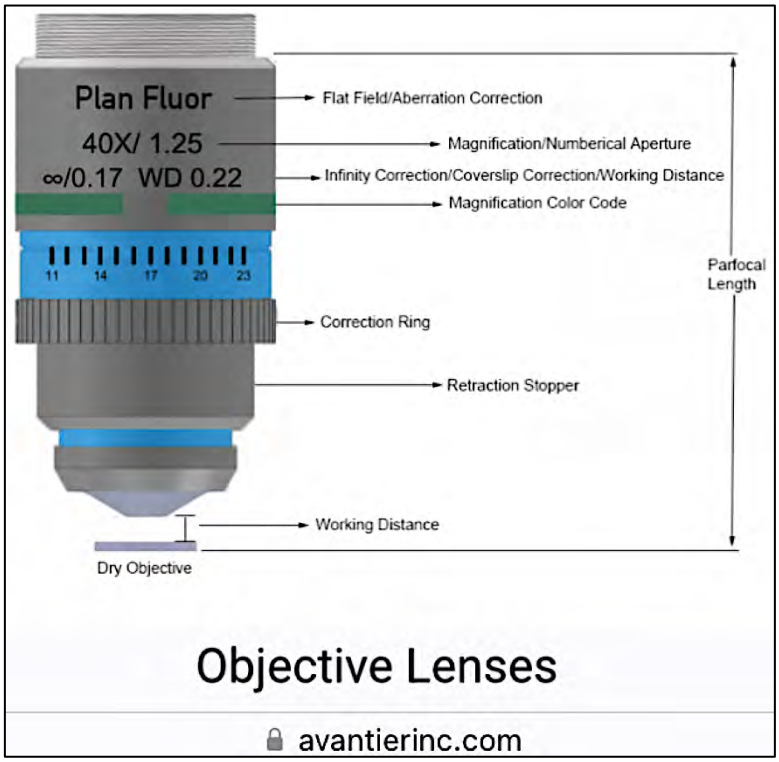
- Stereo microscope: lens characteristics: - look at the numbers in the lens and the knob
  - **Zoom knob/turret:** look at the number in the knob for the magnification factor (Note: magnification is typically continuous. It is key to remember the magnification if you are going to measure specimens)
  - **Objective lens magnification**: look at the number in the lens. The power of the objective lens is typically 1X magnification (also 0.5X, 1.5 X, 2X magnification)

- Compound microscope: Nose piece or revolving turret: holds different lenses in compound microscopes. Lenses are typically arranged in order of magnification (4X, 10X, 20X, 40X, 100X). Rotate the nosepiece (arrow) to bring the desired lens in place (you will hear/fell the click when the lens is in place!). Do not hold onto the objectives to rotate the turret. Always lower the stage before you put the sample/slide and start with 4X objective (*Note: not doing so may cause lenses to break when they come in contact with the slide)*

- Compound microscope: lens characteristics: - look at the words and numbers written in the lens:
  - **Magnification**: the power of the eyepiece; typically, a combination of the following magnifications: 4X, 10X, 20X, 40X, 100X (immersion); in order of increased magnification; they are color coded.
  - **Numerical aperture (**n.a.): Ability to gather light and Resolving power (the higher the number, the more light it gathers and the more detail)



- **Corrections**:
  - Achromatic: most used; correct chromatic aberration in red and blue light and spherical aberration in green
  - Plan Achromatic: + correction of field curvature
  - Plan Fluorite: improved chromatic and spherical aberration; flat field
  - Plan Apochromatic: correct chromatic aberrations in all 3 colors; correct spherical aberrations in 2-3 wavelengths
- **Cover glass**: corrected for specific thickness (standard is 0.17 mm)
- **Immersion media**: used to minimize refractive index between objective and sample (e.g., oil)

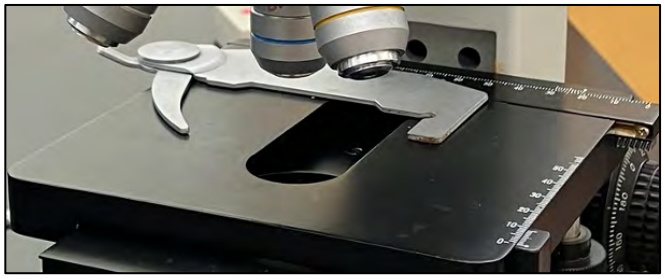



- Cleaning objective lenses: use lens paper. No chemicals, not cloths,…

**TOTAL MAGNIFICATION:**

- Compound microscope: Multiply the eyepiece lens magnification by the objective lens magnification (e.g., 10X x 4X = 40X)
- Stereomicroscope: Multiply the eyepiece lens magnification by the objective lens magnification by the zoom magnification (e.g., 10X x 1X x 3X = 30X)

**STAGE or PLATFORM:**



- Where you place the specimen/slide
- Can have clips to hold slide in place
- In compound microscopes the height of the stage can be adjusted.
- Aperture in compound microscopes: the hole in the middle of the stage that allows the light to pass
- Always lower the stage before you put the sample/slide and start with 4X objective

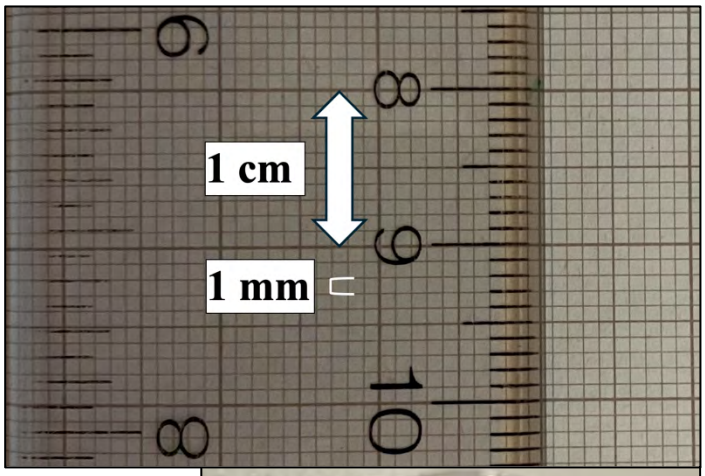
**COARSE AND FINE ADJUSTMENT KNOBS:**


- Used to focus/sharpen the image
- In stereomicroscopes: knobs lower or raise the body of the microscope
- In compound microscopes: knobs lower or raise the stage where the slide is placed.
  - Use first the **coarse** knob for low magnifications then the **fine** knob to obtain a clear image.
  - As you change to higher magnifications, the image will be a bit blurry. You should **always** use the **fine** knob to get a clear image at higher magnifications (never the coarse one as this may result in breaking the lenses)

**ARM:**
Only part of the microscope that you can use to hold/transport it.

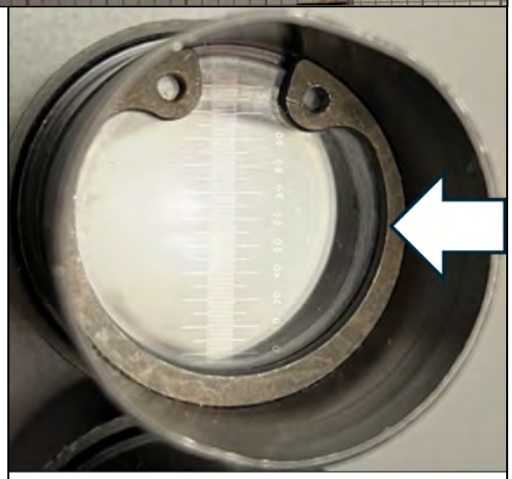
**OCULAR MICROMETER CALIBRATION**:

- Compound microscope: use a stage micrometer (this is a microscope slide with a precise scale)
- Stereo microscope: you can use a stage micrometer and also millimetric graph paper (see figure)




- Compare the stage microscope (or millimetric graph paper) scale to the ocular micrometer scale to determine the length of a division of the ocular micrometer scale.
  - this will depend on the magnification of the objective so, the length of a division in the ocular micrometer needs to be determined for each magnification.

- For spores, measurements are best made in water. To observe ornamentation in spores (e.g., *Russula* and *Lactarius*), use Melzer's reagent. Melzer's gives two types of iodine reaction, either amyloid (grey/blue) or dextrinoid (brown)



**Ocular micrometer**

*Other microscope parts:*

**BASE:**
Holds the light

**CONDENSER:**

- Lens that condenses the light and focuses it on the stage
- The higher the magnification of the condenser, the sharper the image
- Could be moved up and down to control the focus of light

**FIELD DIAFRAGM or IRIS:**
Adjusts the amount of light that reaches the slide.

**MIRROR:**
In stereomicroscopes, it is used to reflect the light from bulbs on the back of the microscope

**LIGHT SOURCES:**
Brightness can be adjusted

**THE HISTORY OF FUNGAL IDENTIFICATION** (this page was developed with Ellen Penso)

Hand drawing, photography, microscopy, electron microscopy, DNA sequencing.

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## TERMINOLOGY

**Fungus** –A eukaryotic organism (having a nucleus and other organelles) with chitin containing cell walls, that need to absorb nutrients from external sources. (Fungi-plural)-This Kingdom includes yeasts, molds and mushrooms. These organisms are mainly decomposers but can have symbiotic relationships with other organisms.

**Ascomycota** – One of the subdivisions (Phyla) of the Kingdom Fungi. This group of mushrooms produce spores in a sac (an ascus) containing 4-8 spores per sac. Examples – morels, cup fungi, *Xylaria* (dead man's fingers).

**Basidiomycota** – One of the subdivisions (Phyla) of the Kingdom Fungi. They produce spores in appendages (sterigmata) that project from club shaped structures (basidia) where meiosis occurs to produce basidiospores. Examples – gilled fungi, boletes, polypores, toothed fungi.

**Mycelium** - The root-like structure which is the mass of branching hyphae.

**Sclerotium** - A compact mass of hardened mycelium (e.g., the interior of a puffball).

**Hyphae** – (Hypha-singular) The filaments produced by spores that release enzymes and chemicals which dissolve organic matter to generate food for the fungus and, at times, for other organisms (e.g., plants).

**Hymenium** - The spore producing tissue layer where cells develop into asci (ascus -singular) or basidia (basidium- singular). This layer can be gills, pores, tooth-like structures or pits.

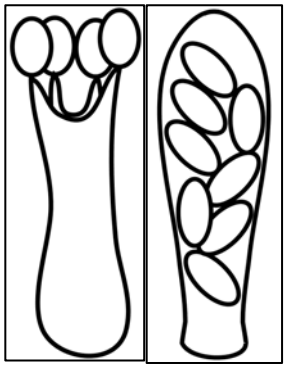
**Stipe or stem or stalk:** structure that supports the **cap** (**pileus**) and connects it to the mycelium

**Spore** – The reproductive unit of fungi which form the hyphae filaments. Spores are dispersed in several ways: Bird's nest fungi by raindrops, stinkhorns by sticking to insects, puffballs by ejection, others by decay into the soil, many by wind.

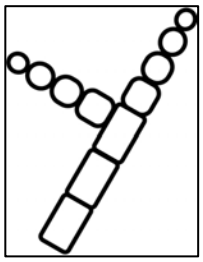
**Ascospores** – Sexual spores from the Ascomycota Phylum (sac fungi) produced inside an **ascus**

**Conidiospores**- Asexual spores produced in **conidia** – (Conidium-singular) which are found in hyphae of the Ascomycota Phylum

**Hyphae** forming **Mycelium**



**Ascus** (left) and **Basidium** (right)



**Conidium**

**Basidiospores** - Sexual spores from the Basidimycota Phylum (sac fungi) produced inside a **basidium**

**Cystidia** – (Cystidium-singular) Large cells in between basidia, edge or face of lamella, surface of the cap or stipe. Often unique to a genus or species (used for identification).

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