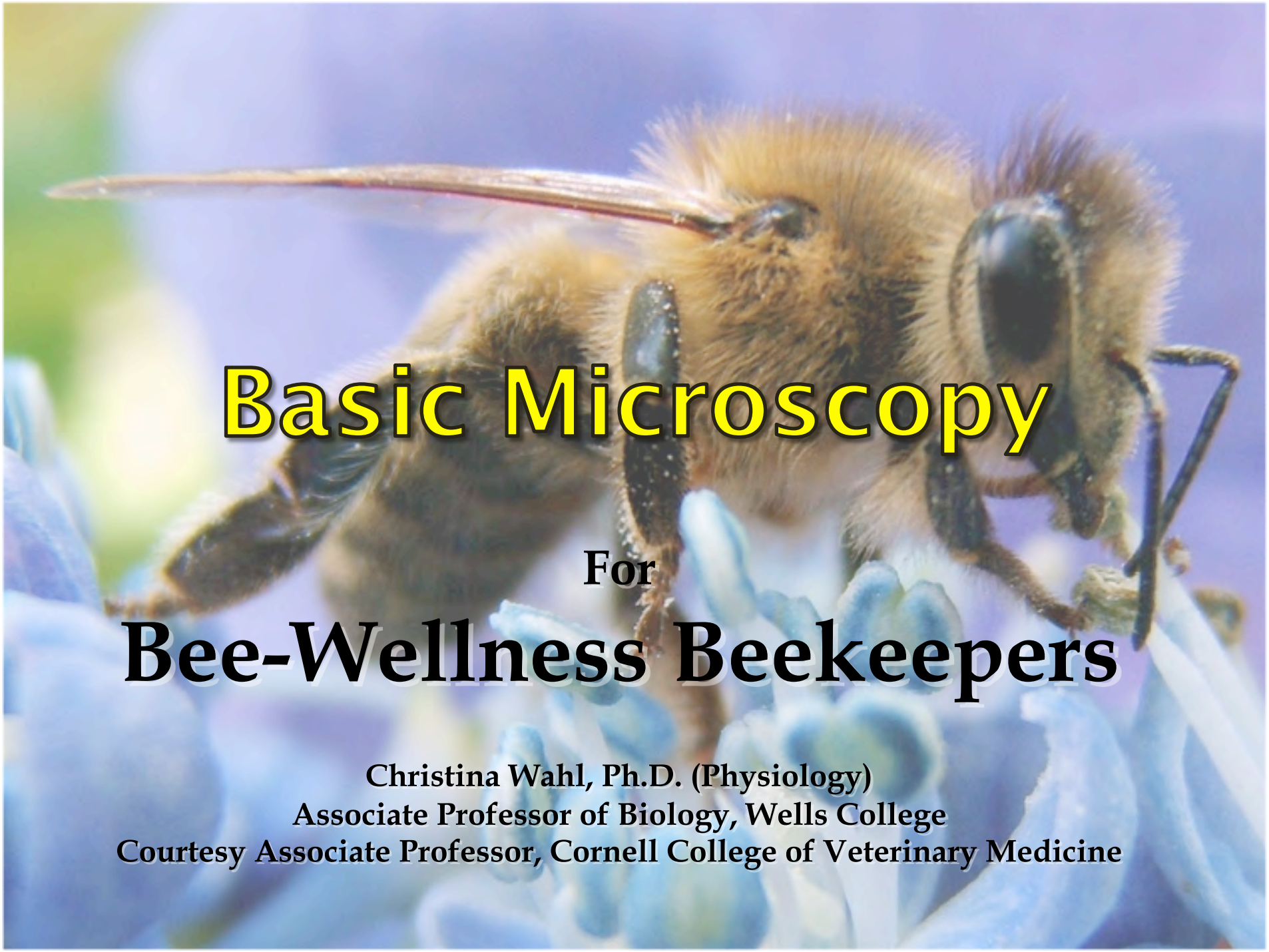

Basic Microscopy

For

Bee-Wellness Beekeepers

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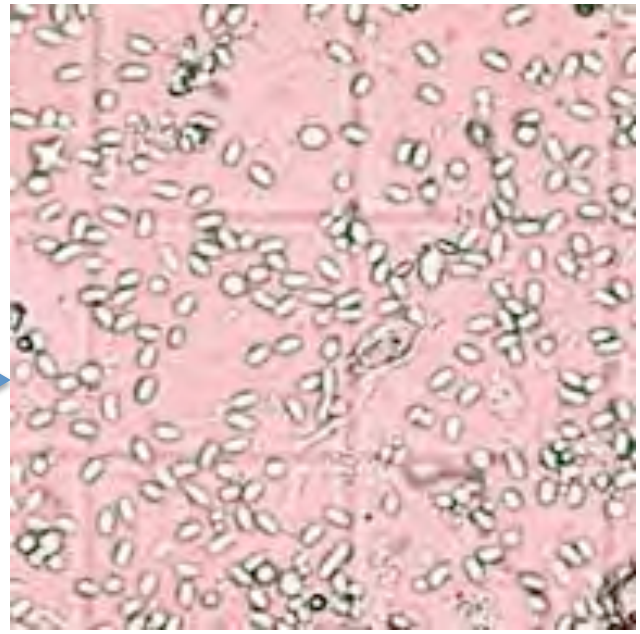
Courtesy Associate Professor, Cornell College of Veterinary Medicine

Overview

- How the microscope can be useful in better understanding the anatomy of your bees and their diseases
- "Dissection" versus "compound" microscopes
  - Microscope "anatomy"
  - How to use the microscope
- How to care for your microscope
- QUIZ yourself!
- Quiz answers

Why do you need a microscope?

What's the matter here? Is it poison? Or does this colony suffer from *Nosema*?



This is *Nosema*! The compound microscope lets you find out.

Why do you need a microscope?

What do my bees look like?

WOW! Check out all those mites!

What you can do with a microscope:

- Dissection type:
  - Learn bee anatomy
  - Examine bees for tracheal mites
  - Look for *Varroa* scars and other injuries to the exoskeleton
  - Study any structure at the *organ* or *whole animal* level.

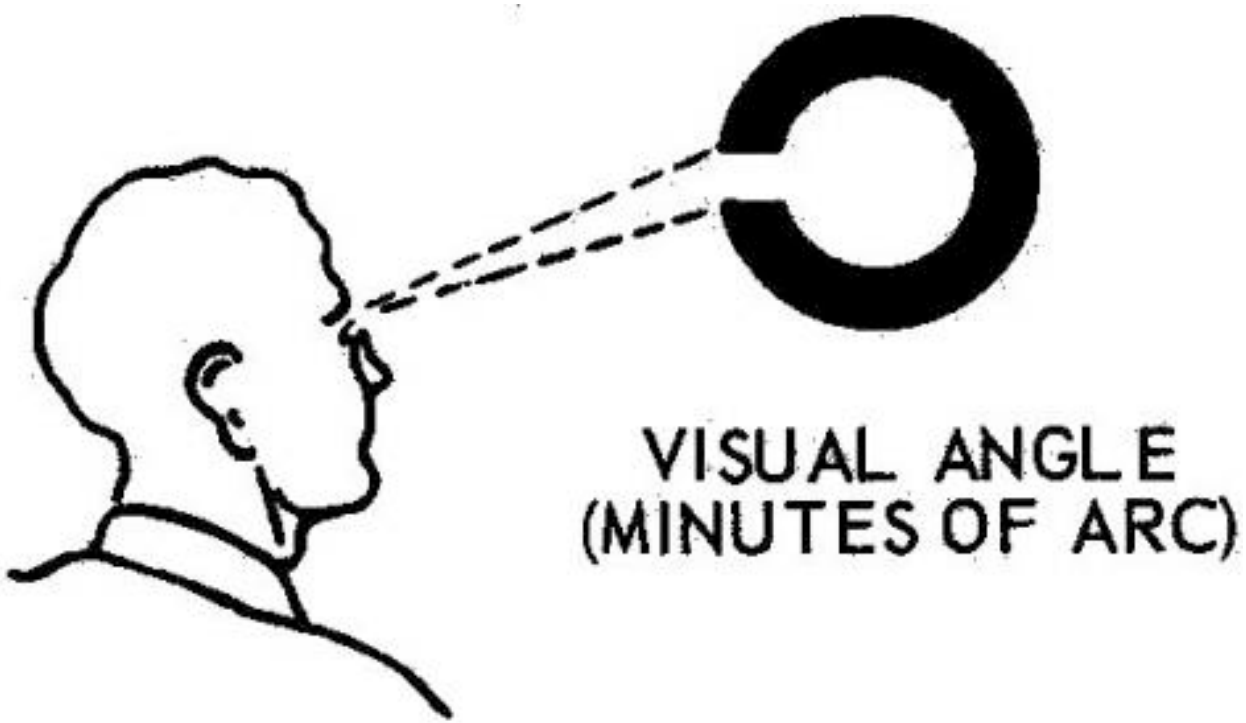
- Compound type:
  - Identify *Nosema* and estimate infection load
  - Identify amoeboid cysts
  - Look at different kinds of pollen
  - Examine any structure at the *tissue* and *cellular* level.

The purpose of the microscope is to improve **resolution**

- This is not the same thing as "to make little things bigger".
- Example: If you have a small photograph and you enlarge it, there is nothing "new" there to see…you have only "made a little thing bigger".
- However, if you get corrective lenses to see more "clearly", you have **improved the resolution** of your eyesight.

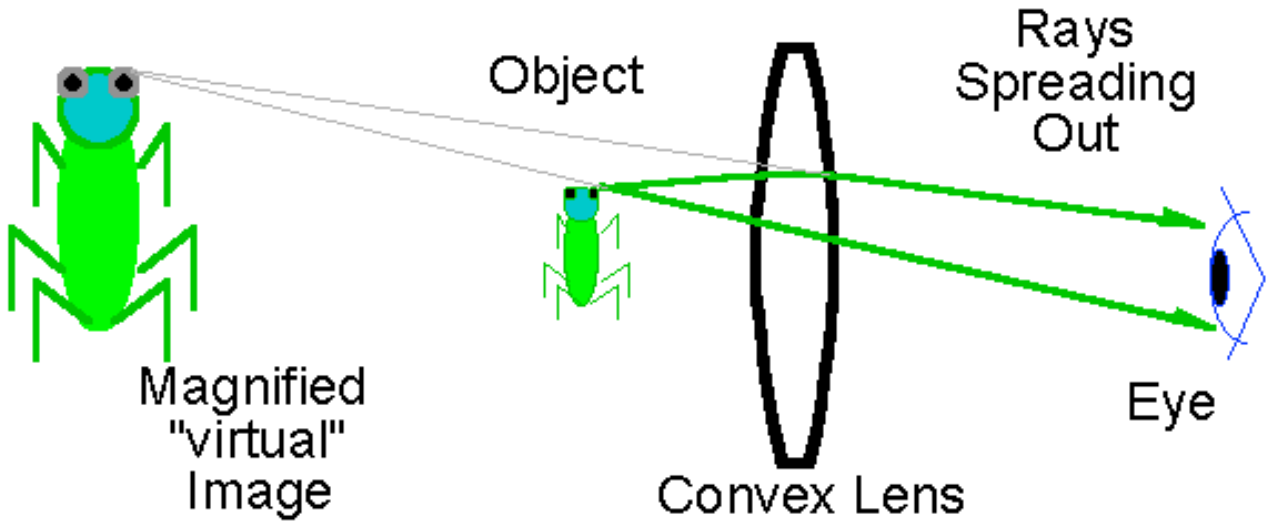
Resolution is defined as the smallest distance between two points at which one can still see two points instead of one.

This is called the
"*minimum separable distance*".



Microscopes improve resolution

using high-quality glass lenses to magnify objects thus increasing the "minimum separable distance" on your retina.



If a convex lens is held near an object, the object looks bigger because the lens bends the light rays inward. The eyes trace the light rays back in straight lines and see a "virtual" image that is magnified.
(Description from *Light* by David Burnie)

Resolution is IMPROVED by:

- ✓Better lighting
- ✓Higher quality lenses
- ✓Stronger lenses (greater magnifying power)
- ✓No dirt in the light path between your eye and the object you are looking at
- ✓Uniform wavelengths from the light source (less color range, or frequency)

Resolution is DEGRADED by:

☹Dirt on the lenses or other surfaces light passes through between the object you are looking at and your eye

☹ Dim light

☹ Highly scattered, broadband light (wide color range)

☹ Poor quality optics or scratched lenses

The Ideal Microscope

- Has high quality optics
- Is well-machined so that it stays in focus when you adjust it
- Has a wide field of view
- Has a versatile range of magnifications
- Has a well-designed stage (place to put your specimen)
- Is easy to use

Important Features of the Dissection Microscope

- ✓ Good depth of field or "working distance".

  **This means that the 3-D contours of the specimen are all in focus at the same time over a generous observation "depth".

- ✓ Allows manipulation of the specimen while you are looking at it.

Optional, but handy features in a dissecting scope

- Binocular…both eyes can observe the specimen
- Two light sources: One above, and one below your specimen. These can be used together, or one at a time, and the intensity of the light may be increased or decreased.
- LED light source is cool (doesn't heat the specimen) and uses little power.
- Zoom lens allows you to choose exactly the magnification that works best for what you are doing.

The Dissecting Scope

This is a Zeiss Stemi DV4

Binocular

zoom lens adjustment

focusing knob

adjustable illumination from the *top, bottom, or both*

It has three-dimensional, relaxed stereo viewing from 8-32x power with a 92mm depth of field.

The Compound Microscope

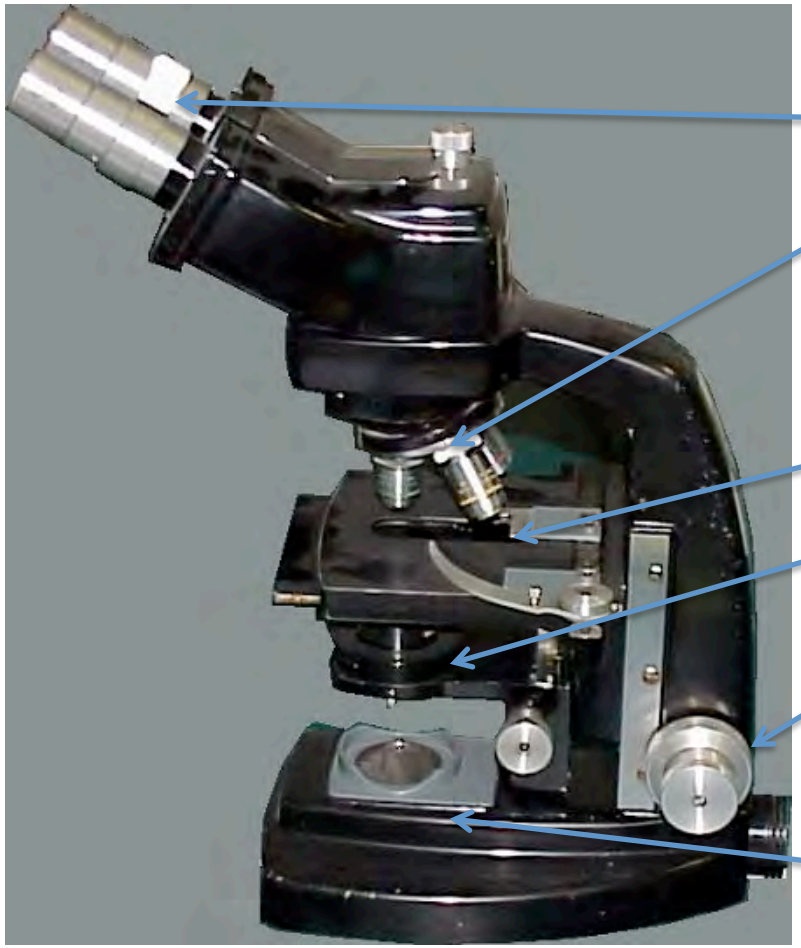
Important features:

- ✓ Has enough objective lenses to allow you the range of magnifications you need
- ✓ Has high quality, wide field of view optics
- ✓ Has a stable, tight stage that doesn't drift out of focus
- ✓ Has an adjustable sub-stage condenser to allow you to direct and concentrate the light where you need it

Optional, but handy features in a compound scope

- Binocular….both eyes can observe the specimen at once. The ocular lenses are independently adjustable to accommodate intraocular disparities of the observer.
- Clips on the stage hold the specimen slide, and two worm gears allow precise movements of the stage, smoothly moving different parts of the slide into view.
- A generous range of objective lenses that achieve several levels of magnification

The Compound Microscope



Bausch & Lomb

Binocular

4X, 10X, 40X, and 100X objective lenses on the objective *lens turret* (revolving nosepiece)

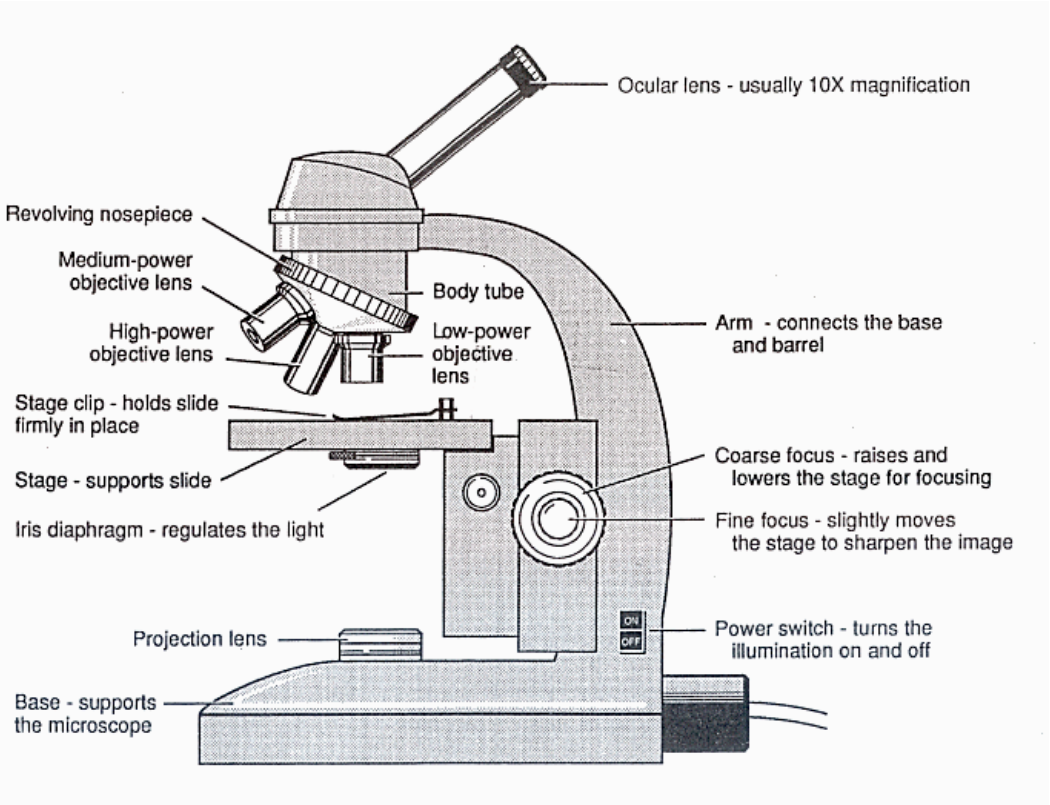
Movable stage with stage clip

Substage condenser with diaphragm

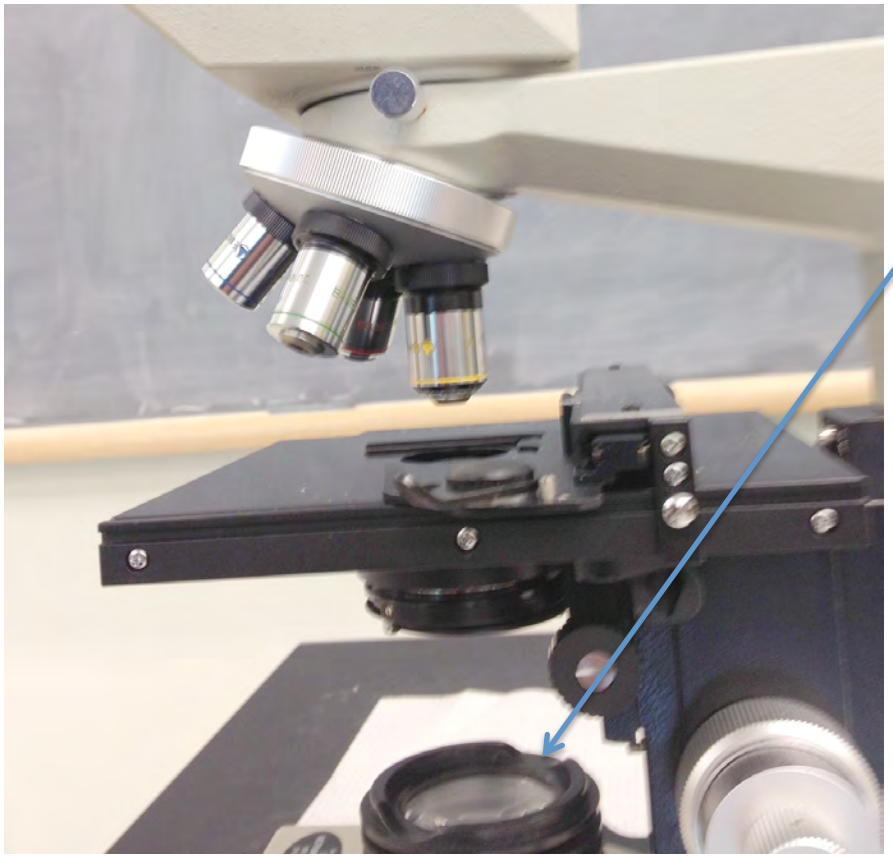
Coarse and fine focusing knobs

Field condenser

The Compound Microscope has More Moving Parts than a Dissecting Scope



This is a close-up of the lens turret and stage. The lens you need can be rotated into position on the turret. The clip holds the slide in place. The hole in the stage allows light to shine up from below, through the *substage condenser*.



- Here you can see the field condenser lens at the bottom of the photo. The lamp is just under it, and the light comes up through several lenses, through the stage, through the slide, through the objectives, through the body tube, through the oculars, to your eyes.

- The more lenses that are built into a microscope, the worse the resolution, because optical aberrations occur at the interfaces. The best compound microscopes have the fewest pieces of glass between the specimen and your eyes.

Basic rules of use:

- Don’t touch the lenses
- Carry the microscope by the base and body
- Keep the stage clean
- Use ONLY lens paper to clean the lenses, and a small amount of alcohol or water.
- Get professional servicing if the instrument is damaged, if you can’t clean the lenses of dirt, or if the light path is out of alignment.

Basic operation:
Steps to focus on the slide:

1. Turn on the light source.
2. Place your slide into the clip on the stage
3. Always begin to focus your specimen using the lowest power objective.
4. Never try to do your initial focus while looking through the oculars. Always look directly at the stage at first, so that you don't accidentally jam the objective lens into your slide specimen. Raise the stage until the objective is close to the specimen, then look through the oculars and lower the stage until the object is in focus.
5. Center and focus the substage condenser (to be demonstrated)
6. Adjust the substage diaphragm (to be demonstrated)
7. Increase magnification, refocus, and readjust the substage diaphragm as needed.

Quiz.

1. What is resolution?
2. What is the best type of microscope to look at bacteria or internal parasites?
3. What type of instrument can I use to look for *Varroa* scars on my bees?
4. After I turn on the microscope light source and place a slide specimen on the compound stage, what is the first step to follow in bringing the object into focus?

Answers

1 What is resolution?

The minimum distance at which you can distinguish between two points.

2 What is the best type of microscope to look at bacteria or internal parasites?

The compound microscope

3 What type of instrument can I use to look for *Varroa* scars on my bees?

The dissection microscope

4 After I turn on the microscope light source and place a slide specimen on the compound stage, what is the first step to follow in bringing the object into focus?

While looking directly at the stage (not through the oculars!) bring the stage up close to the objective. Then focus by moving the stage AWAY from the objective while looking through the oculars.

Questions

5 What are two important features of a dissection scope?

6 What are two important features of a compound scope?

7 How do I clean the lenses in any microscope?

8 When do I need to have the microscope professionally serviced?

Answers

5 What are two important features of a dissection scope?

Good depth of field. Allows manipulation of the specimen while viewing through the oculars.

6 What are the important features of a compound scope?

- ✓ *Has enough objective lenses to allow you the range of magnifications you need*
- ✓ *Has high quality, wide field of view optics*
- ✓ *Has a stable, tight stage that doesn't drift out of focus*
- ✓ *Has an adjustable sub-stage condenser to allow you to direct and concentrate the light where you need it*

7 How do I clean the lenses in any microscope?

Use LENS PAPER and a small amount of water or alcohol

8 When do I need to have the microscope professionally serviced?

If the instrument is damaged, if you can't clean the lenses of dirt, or if the light path is out of alignment

Acknowledgements

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