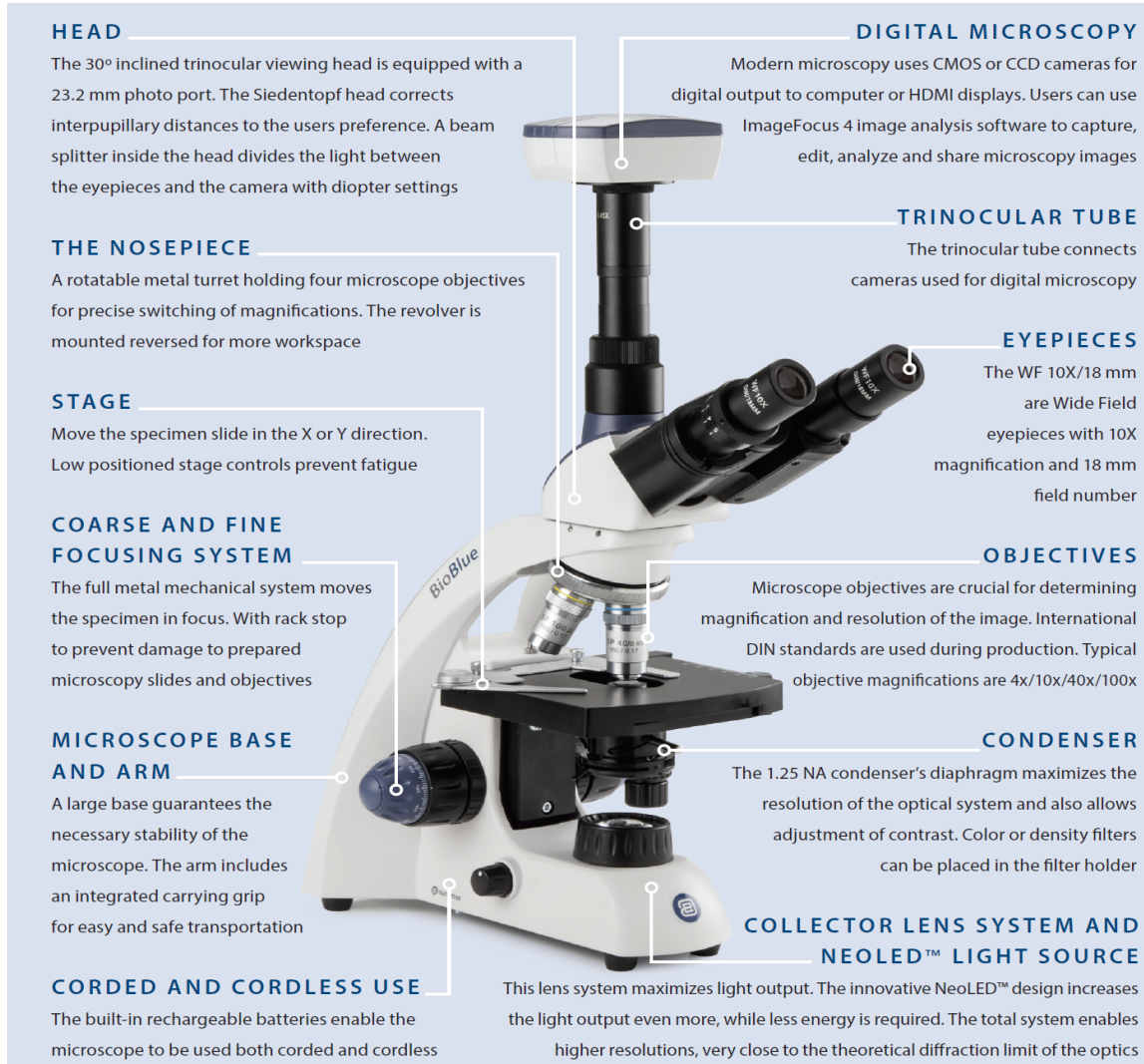


## BRIEF INTRODUCTION

### About Microscopy

A compound microscope consists out of a group of lenses (called objective) which focuses a real image of the object inside the microscope. A second group of lenses (called eyepiece) magnifies this image and projects it on the retina of the eye. This compound optical system (objective - eyepiece) together with other mechanical components are the basics of a modern microscope



### Objectives

Most common microscope objectives come in three different types: achromatic, semi plan and plan

**Achromatic objectives:** Usually contain a pair of lenses and correct for colour and have a flat field correction for about 65% of the image. If there are aberrations they occur in the outer 35% of the image

**Semi plan objectives:** Contain three or more (achromatic) lens elements and have an 80% flat field. Semi plan objectives have an improved resolution

**Plan objectives:** Correct even better for colour and spherical aberration than semi plan objectives. Plan objectives have a 95% flat field and produce the best image quality



Fungus

### Anti-fungus treated

Fungus spores are parasitic, travel in the air and can settle inside lenses. High temperatures, humidity and environments that are dark and unventilated encourage fungus growth

The optical components are anti-fungus treated.

Nevertheless it is best to minimize the possibility of fungus by storing the microscopes in well-ventilated rooms with moderate temperatures and low humidity.

### Resolving power

A human eye can not distinguish two points when these are closer than approximately 0.3 mm or 300  $\mu\text{m}$  to each other. That is what is called “resolving power”, the capacity to resolve 2 two points which are close together or “optical resolution”, the ability of an imaging system to resolve detail

### Magnification

First of all the client should tell us the size of the sample to be observed. Let’s imagine we have a few particles of 10 microns. In order to see in the microscope eyepiece these particles without problems, we need at least 100 magnification.

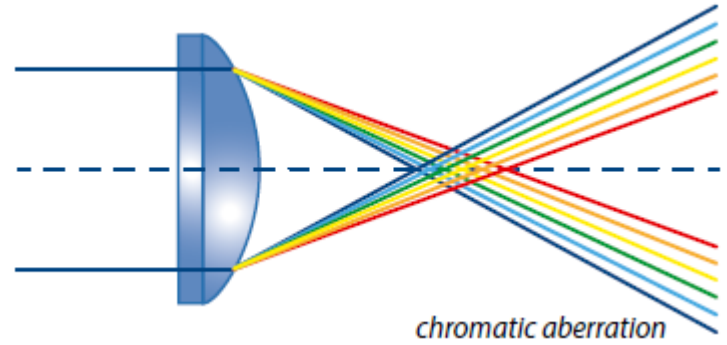
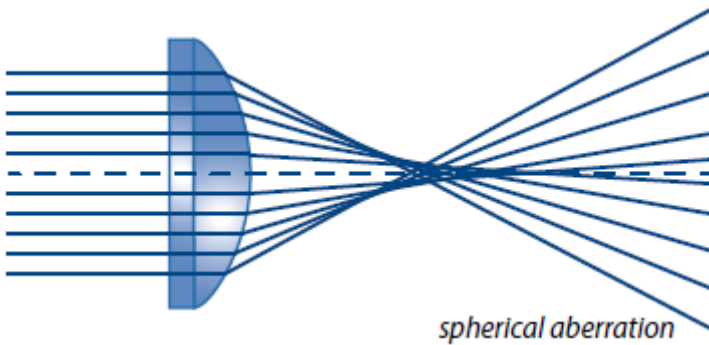
$0.010 \text{ mm} \times 100 = 1 \text{ mm}$ .

At 100x the customer will see this particle of 1 mm in the field of view of the eyepiece

### Optical resolution

The resolution of optical microscope can be defined as the shortest distance between two points that the microscope can reveal for a human eye. The smaller this value, the higher the resolving power of the microscope and the better the clarity and detail of the image. If two bacterial cells were very close together on a slide, they might look like a single, blurry dot on a microscope with low resolving power, but could be told apart as separate on a microscope with high resolving power.

However, resolving power is ultimately limited not by microscope machining quality, but by the physical properties of light. If two structures are separated by a distance less than half the wavelength of the light used for imaging, they cannot be distinguished from each other by conventional light microscopy. This phenomenon is called the diffraction barrier.



### Aberrations

In practice, the resolution of a microscope is lower than the theoretical resolution due to optical aberrations, Improper illumination or bad alignment of the optics and components of the microscope. Despite corrections for chromatic and spherical aberrations, coma, astigmatism and distortion, the real maximum resolution of a microscope with a 100x objective is about 0.25  $\mu\text{m}$  for 550 nm light rays

**Chromatic aberration** Difference in focus of different colors caused by difference in refractive indices for each wavelength

**Spherical aberration** Difference in focus caused by defects or irregularities on the surface of a lens

**Astigmatism** Rays traveling through two perpendicular planes having different focus

**Coma aberration** Variation in magnification over the entrance pupil

**Distortion** Stretching of an image as a variation in magnification across the lens field

### How do I calculate the total magnification?

Calculation of the magnifications is achieved by multiplying the factors of magnification of the objective (optical component closest to the sample) and the magnification of the eyepiece (optical component by which we observe). For example:

Biological microscope with 10x eyepiece and 40x objective. Total magnification  $10 \times 40 = 400\text{x}$ .

### Which is the maximum magnification that can be achieved?

The limit of visual magnifications with resolving power in optical microscopes is usually 1250 to 1300 total magnifications.

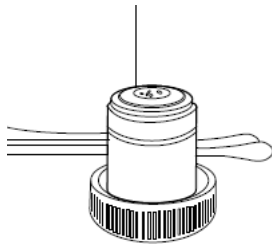
Above these increases the image would look bigger but no more defined (resolved).

## HOW TO USE A COMPOUND MICROSCOPE

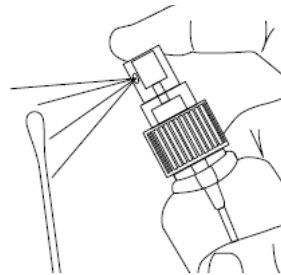
- Set your microscope on a flat, sturdy surface. Plug the microscope's power cord into an outlet.
- Switch on your microscope's light source and then adjust the light adjustment knob until the illumination is comfortable for observation
- Rotate the nosepiece to the lowest-power objective (usually 4x for 40x magnification).
- Place a microscope slide on the stage, either under the stage clips or clipped onto the mechanical stage if your microscope has one.
- Adjust the coarse focus knob until the specimen is in focus. Slowly move the slide to center the specimen under the lens, if necessary. Do this by nudging it gently with your fingers or by the slide control knobs if you have a mechanical stage.
- Adjust the fine focus knob until the specimen is clearly in focus. Then adjust the diaphragm to get the best lighting. Start with the most light and gradually lessen it until the specimen image has clear
- Rotate the nosepiece to the 10x objective for 100x magnification. Refocus and view your specimen carefully. Adjust the lighting again until the image is most clear (you will need more light for higher power). Repeat with the 40x objective for 400x magnification.
- Optional: If your microscope has a 100x oil-immersion lens, you'll need to put 1-2 drops of immersion oil over the slide coverslip (the piece of glass over the middle of the slide). Move the 100x objective lens into position, and then slowly move the stage up until the lens makes contact with the oil. With the 100x lens, you will be able to see additional cell detail. When you are done using the slide, clean the oil off of the slide and the lens with lens cleaning paper and solution.

### How to keep the optics clean?

Keeping the optical system of your microscope clean is essential for the best image quality and overall lifetime of your microscope.



**1** Place your objective or eyepiece on a secure location. Objectives can be screwed into the cover of an objective case, eyepieces can be placed in the microscope box. Condensers and collector lenses can stay in their place in the microscope



**2** To prevent scratches on coatings and optical glass, try to remove dirt and dust that sticks to the optical surface first with an air-blower or pressurized dry air (oil-free and under moderate pressure version only)



**3** Use an absorbent lens paper or cotton swap. Damp a swap or towel with a small amount of lens cleaning fluid or cleaning mixture (Either pure iso-propanol or a mixture of 7 parts ether and 3 parts alcohol)



**4** Clean the lens by using the tip of the cotton swap or the lens paper. When cleaning a large lens surface, wipe with little pressure from the centre towards the periphery in a circular motion, do not use zig-zag motion. Discard after a single use



**5** Wait until the cleaning fluid is vaporated, or speed up this process by using pressurized dry air

**6** Check and return. With the help of a magnifying glass check if the surface is clean and place the cleaned item back on the microscope



## BRIEF INTRODUCTION About Stereomicroscopes

A stereo, or dissecting, microscope provides a three-dimensional view of the specimen. It does this with separate objective lenses and eyepieces for each eye. They have lower magnification when compared to compound microscopes, but they also have a longer working distance.

### How Does a Stereo Microscope Work?

A stereo model is an optical microscope that functions at a low magnification. It works by using two separate optical paths instead of just one. The two objectives and two eyepieces provide the eyes with slightly different viewing angles. In essence, the left and right eye are seeing the same object but in a different way.

Much like what happens with your eyes, these two separate viewing angles yield a three-dimensional image. This feature makes it ideal for examining surfaces of solid materials. It also lends itself well to sorting and dissecting. One can use these units for working with watches, circuits and three dimensional samples.

### STEREO OR BIOLOGICAL MICROSCOPE?

What is the difference between a stereo microscope and a biological microscope? And why would you choose to purchase a stereo microscope instead of a biological microscope? Let's take a look at the differences between the two types of microscopes.



Take a look at the **biological microscope** shown above and notice these features:

The stage only has room on it for microscope slides, no room for large or bulky specimens.

The only light on a biology microscope comes from beneath the stage.

The magnification of a biological microscope is much higher than that of a stereo microscope.

Magnification is changed by revolving the nosepiece (either 4x, 10x, 40x or 100x).



The **stereo microscope** shown above has several features that are not available on a biological microscope:

Plenty of working area for larger objects such as rocks, flowers, large insects, metal parts, etc.

The light comes both from above and beneath the stage.

The knob on the side of the head allows zoom magnification between approx 6x - 45x, which means you can see 7x, 8x, 9x, etc.

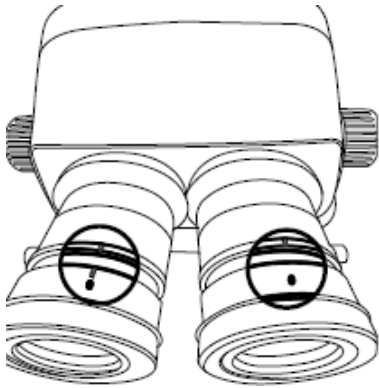
In conclusion, depending on what type of sample you need to view will determine whether a stereo microscope or a biological microscope is better for your needs. Some specimens and the microscope best suited to view those items are in the below table.

Biological microscope	Stereo microscope
Blood cells	Flowers
Bacteria	Rocks
Skin cells	Coins and Industrial metal parts
Prepared Slides	Insects

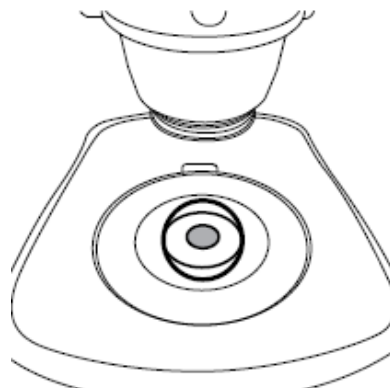


## How to set up a Stereo Microscope

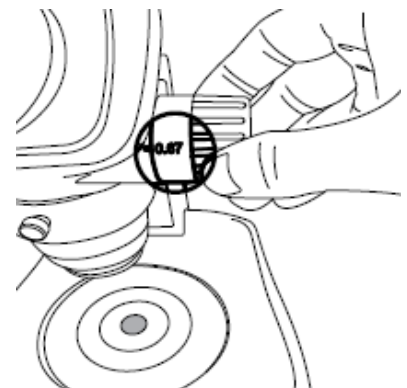
Setting up your stereo microscope properly is essential to getting parfocal images during the entire zoom range. It also prevents head aches, stressful eyes and fatigue. Below you will find a set up guide that will help get the best out of your microscope



**1** Turn the diopter adjustment rings of both eyepieces to position "0"



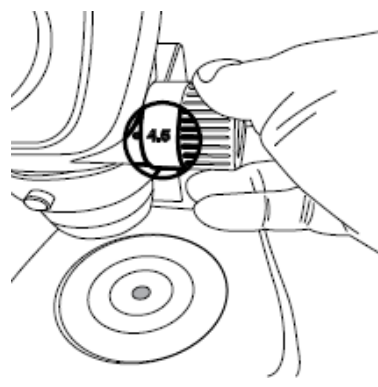
**2** Put a specimen on the stage plate



**3** Turn the zoom adjustment knob to the lowest magnification



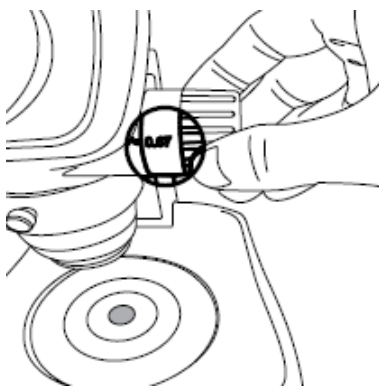
**4** Rotate the focus adjustment knob to bring the specimen into focus



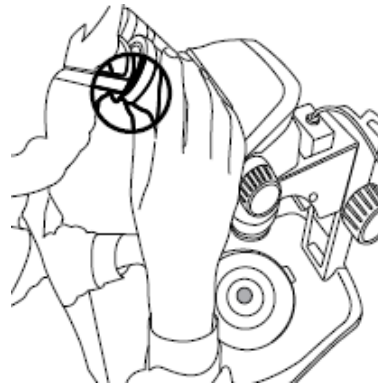
**5** Turn the zoom adjustment knob to the highest magnification



**6** Rotate the focus adjustment knob to bring the specimen into focus



**7** Turn the zoom adjustment knob to the lowest magnification



**8** Rotate the diopter adjustment rings of the left and right eyepiece to bring the specimen into focus

### Please note

Put the zoom adjustment knob at the highest magnification again and check the image focussing. The diopter adjustment is complete when the image is accurately focussed during zooming. If not, please repeat steps 3 to 8