Light Microscopy (contd)

Components of a compound microscope

Eyepiece (ocular lens) (1)

Objective turret, revolver, or revolving **nose piece** (2)

Objective lenses (3)

Focus knobs (to move the stage)
Coarse adjustment (4)

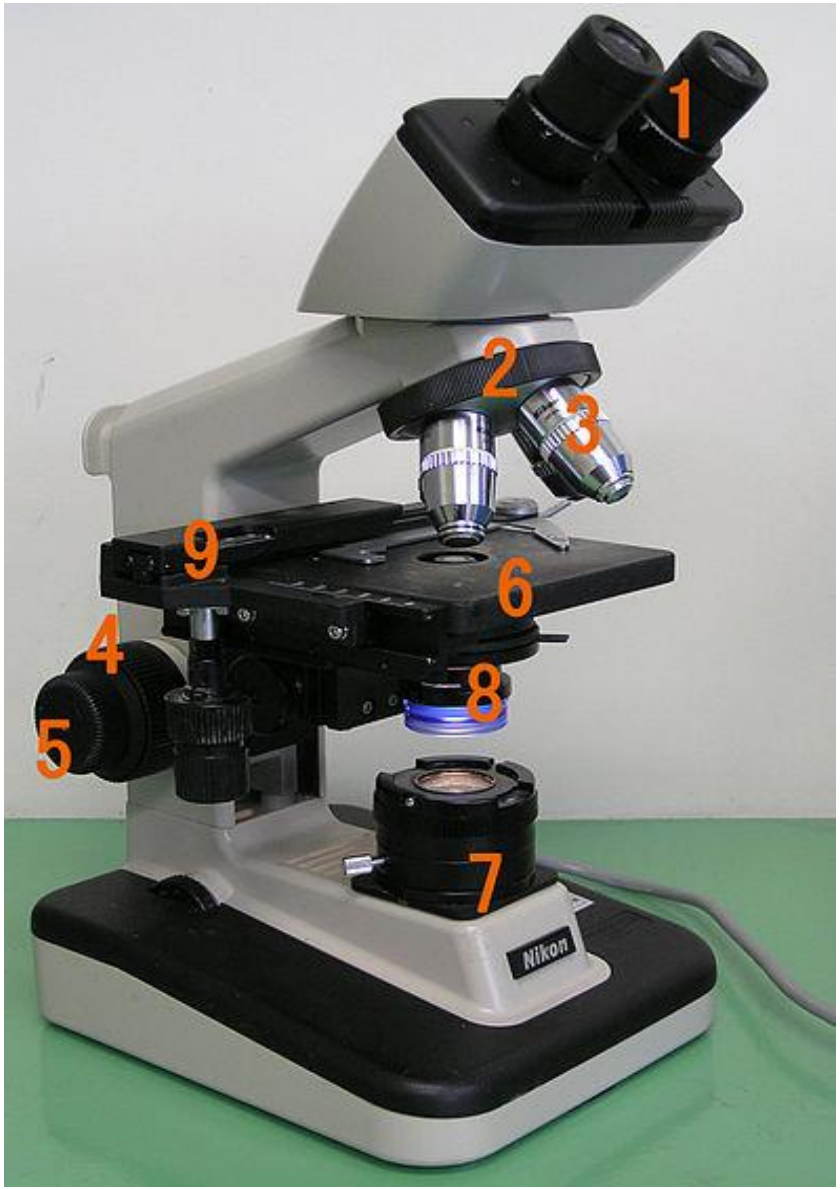
Fine adjustment (5)

Stage (to hold the specimen) (6)

Light source (a light or a mirror) (7)

Diaphragm and condenser (8)

Mechanical stage (9)



Eyepiece (ocular lens)

The eyepiece, or ocular lens, is a **cylinder containing two or more lenses.**

Its function is to **bring the image into focus** for the eye.

The eyepiece is inserted into the **top end** of the body tube.

Eyepieces are **interchangeable** and many different eyepieces can be inserted with different degrees of magnification.

- Typical **magnification values** for eyepieces include **5×, 10× (the most common), 15× and 20×.**

- In some **high performance microscopes**, the optical configuration of the objective lens and eyepiece are **matched** to give the best possible optical performance.

- This occurs most commonly with **apochromatic objectives**.

Apochromatic lens: free from chromatic and spherical aberration

Objectives

At the **lower end** of a typical compound optical microscope, there are **one or more objective lenses** that **collect light from the sample.**

The objective is usually in a **cylinder** housing containing a **glass single** or **multi-element compound lens.**

Typically there are **three or four objective lenses** screwed into a **circular nose piece** which may be rotated to select the required objective lens.

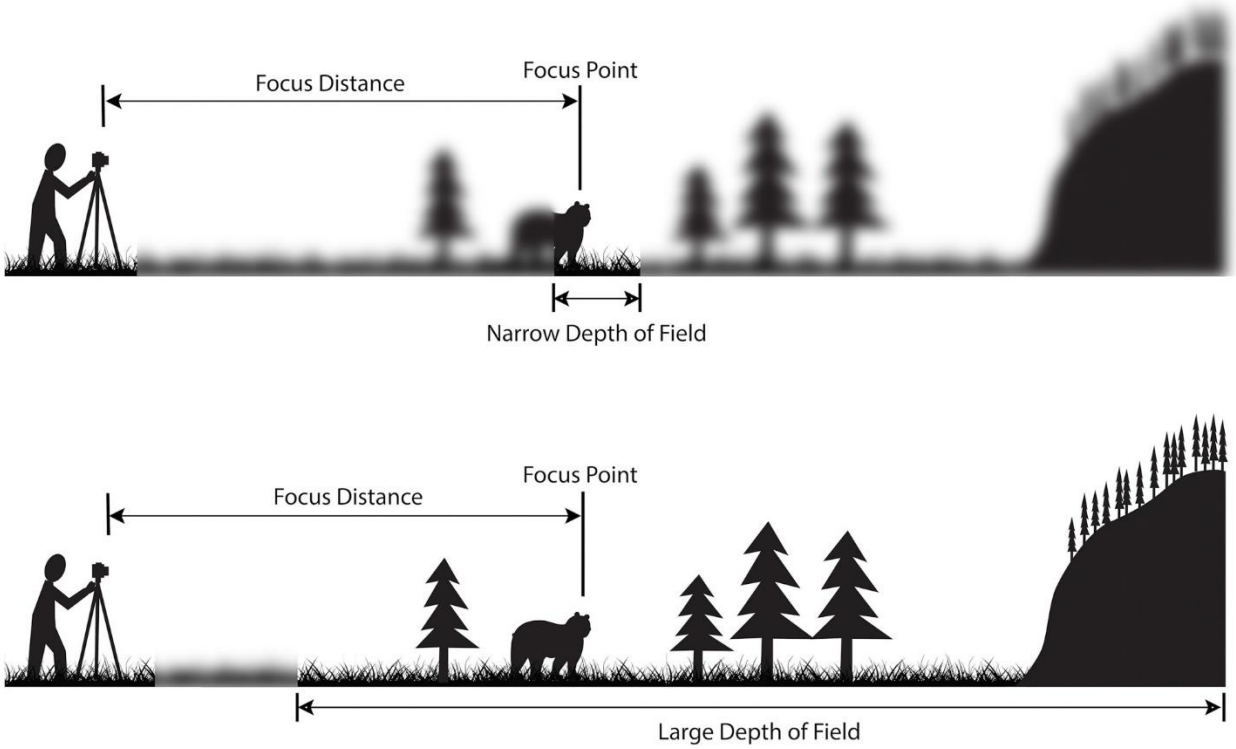
- Microscope objectives are characterized by **two parameters**, namely, **magnification** and **numerical aperture**.

- **Magnification** is the process of enlarging the **apparent** size of some object.

- The **numerical aperture** of a microscope objective is a measure of its **ability to gather light and resolve fine specimen detail** at a fixed object distance.

- The **magnification** typically ranges from **5× to 100×** while the **numerical aperture** ranges from **0.14 to 0.7**, corresponding to focal lengths of about **40 to 2 mm**, respectively.

- Objective lenses with **higher magnifications** normally have a **higher numerical aperture** and a **narrower depth of field in the resulting image.**



- Some **high performance** objective lenses may require **matched** eyepieces to deliver the best optical performance.

Oil immersion objective

- Some microscopes make use of oil-immersion objectives or water-immersion objectives for **greater resolution** at high magnification.

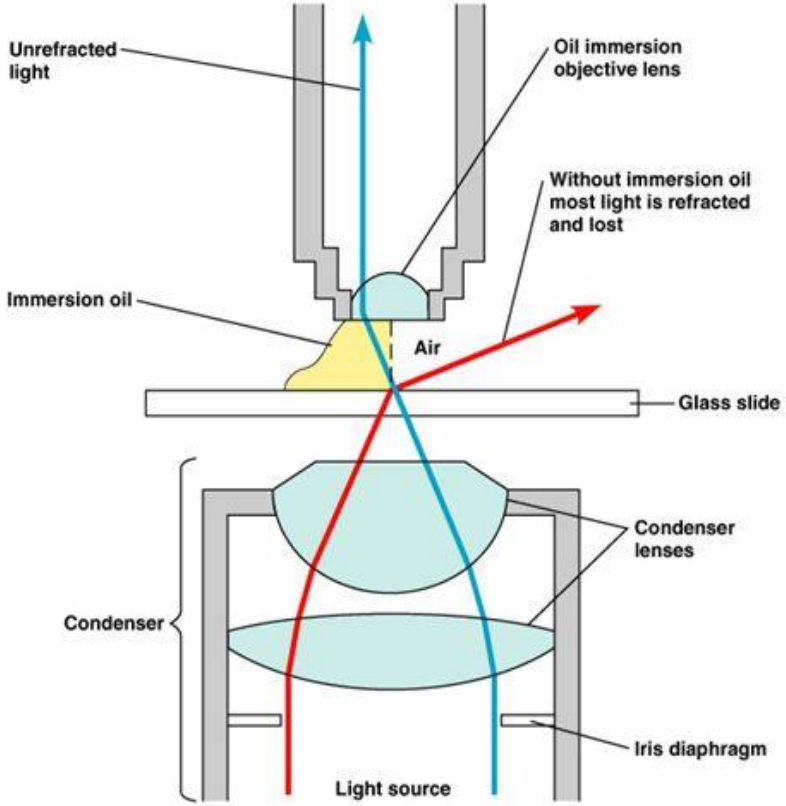
- These are used with **index-matching material** such as immersion oil or water between the objective lens and the sample.

- The **refractive index of the index-matching material is higher than air** allowing the objective lens to have a **larger numerical aperture (greater than 1).**

- So that the light is transmitted from the specimen to the outer face of the objective lens with **minimal refraction**.

Refractive index, also called **index of refraction**, is measure of the bending of a ray of light when passing from one medium into another.

Oil immersion lens



- Numerical apertures as high as **1.6** can be achieved.
- The larger numerical aperture allows **collection of more light** making detailed observation of smaller details possible.
- An oil immersion lens usually has a magnification of **40 to 100×.**

Focus knobs

Adjustment knobs move the **stage up and down** with separate adjustment for **coarse** and **fine focusing.**

The same controls enable the microscope to adjust to **specimens of different thickness**.

Frame

- The whole of the optical assembly is traditionally **attached to a rigid arm**, which in turn is attached to a strong **U-shaped foot** to provide the necessary rigidity.
- The arm angle may be **adjustable** to allow the viewing angle to be adjusted.
- The frame provides a **mounting point** for various microscope controls.

- Normally this will include **controls for focusing**, typically a **large knurled** (having small ridges on the surface for better grip) **wheel to adjust coarse focus**, together with a **smaller knurled wheel to control fine focus.**

- Other features may be **lamp controls** and/or **controls for adjusting the condenser.**

Stage

- The stage is a **platform** below the objective which supports the specimen being viewed.
- In the center of the stage is a **hole** through which light passes to illuminate the specimen.
- The stage usually has **arms to hold slides**.
- At magnifications higher than 100×, moving a slide by hand is not practical.

- A **mechanical stage**, typical of medium and higher priced microscopes, allows tiny movements of the slide via control knobs that reposition the sample/slide as desired.

- If a microscope did not originally have a mechanical stage it may be possible to add one.

- All stages move **up and down** for focus.

- With a mechanical stage, slides move on **two horizontal axes** for positioning the specimen to examine specimen details.

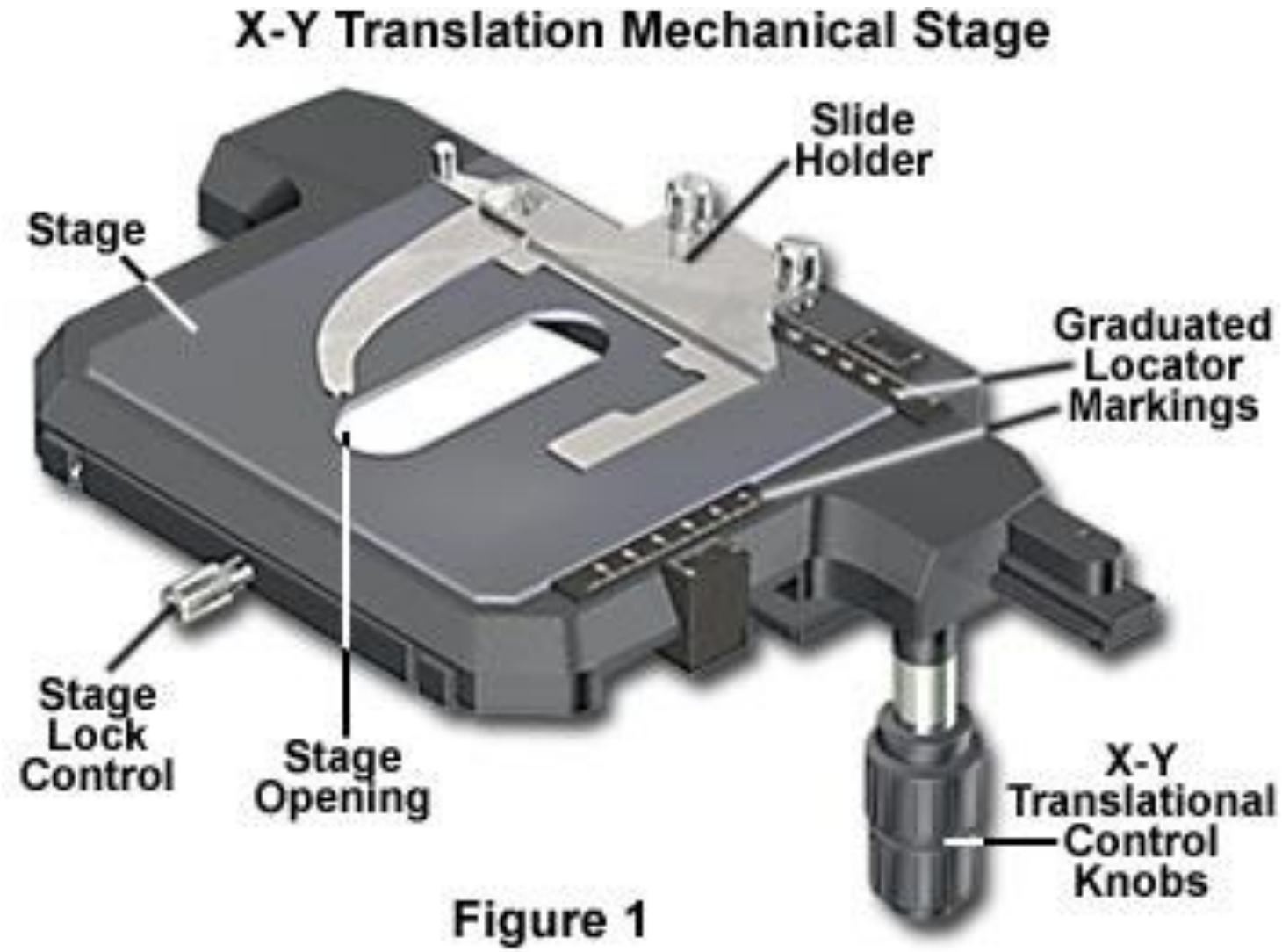

X-Y Translation Mechanical Stage
Slide Holder
Stage
Graduated Locator Markings
Stage Lock Control
Stage Opening
X-Y Translational Control Knobs

Figure 1

- **Focusing starts at lower magnification** in order to center the specimen by the user on the stage.

- Moving to a higher magnification requires the stage to be moved **higher vertically** for re-focus at the higher magnification and may also require slight horizontal specimen position adjustment.

- Due to the difficulty in preparing specimens and mounting them on slides, it's best to **begin with prepared slides** that are centered and focus easily regardless of the focus level used.

Light source

- Many sources of light can be used.
- At its simplest, **daylight** is directed via a mirror.
- Most microscopes, however, have their **own adjustable and controllable light source** – often a **halogen lamp**, although illumination using **LEDs** and **lasers** are becoming a more common provision.
- **Köhler illumination** is often provided on more expensive instruments.

    Köhler illumination generate an even illumination of the sample and ensures that an image of the illumination source is not visible in the resulting image.

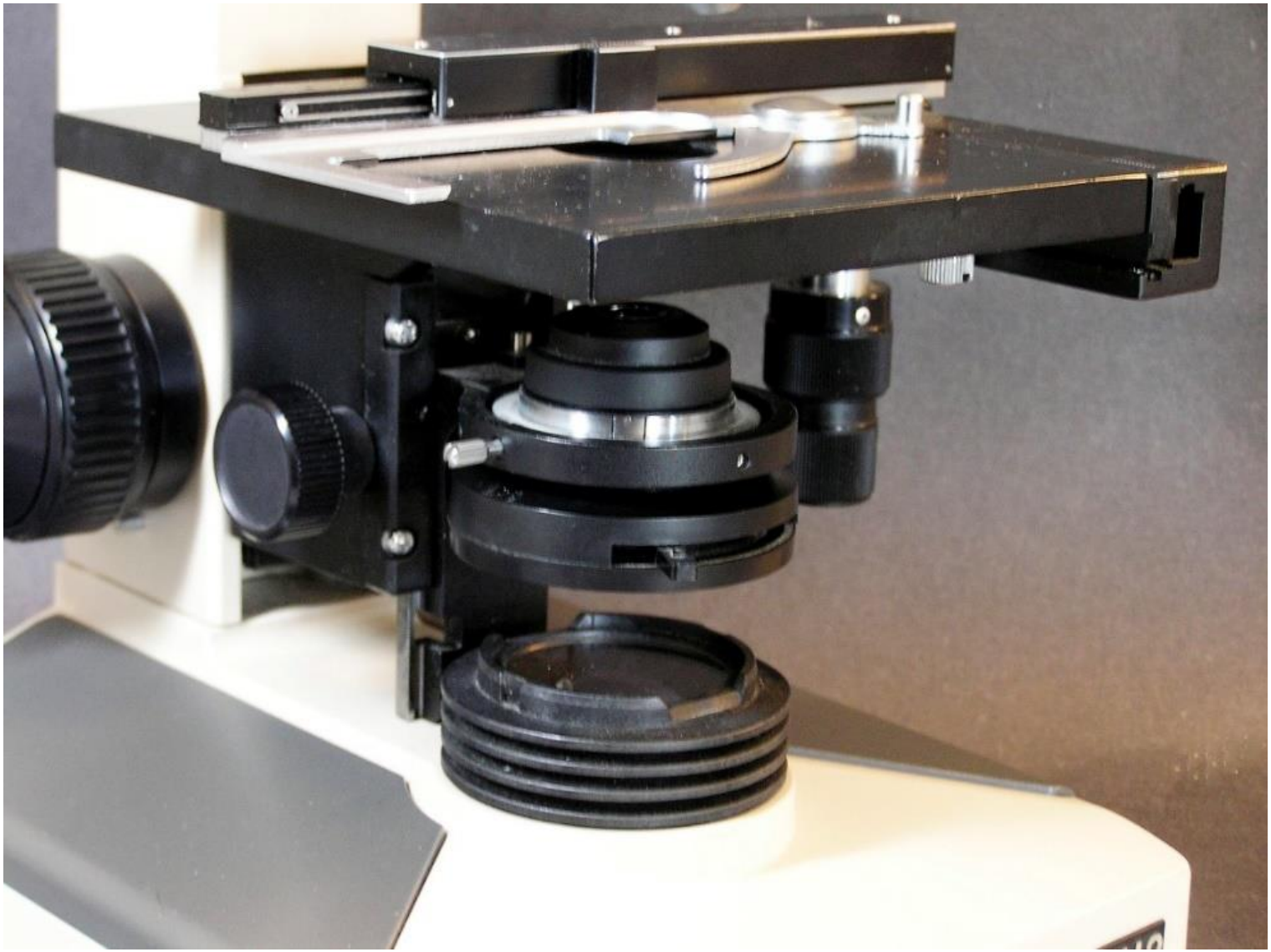

NOVEL

Condenser

- The condenser is a lens designed **to focus light from the illumination source onto the sample.**

- The condenser may also include other features, such as a **diaphragm and/or filters**, to manage the quality and intensity of the illumination.

- For illumination techniques like **dark field** and **phase contrast,** additional optical components must be precisely aligned in the light path.



Magnification

- The actual power or magnification of a compound optical microscope is the **product of the powers of the ocular (eyepiece) and the objective lens.**

- The maximum normal magnifications of the ocular and objective are **10× and 100×** respectively, giving a final magnification of **1,000×**.

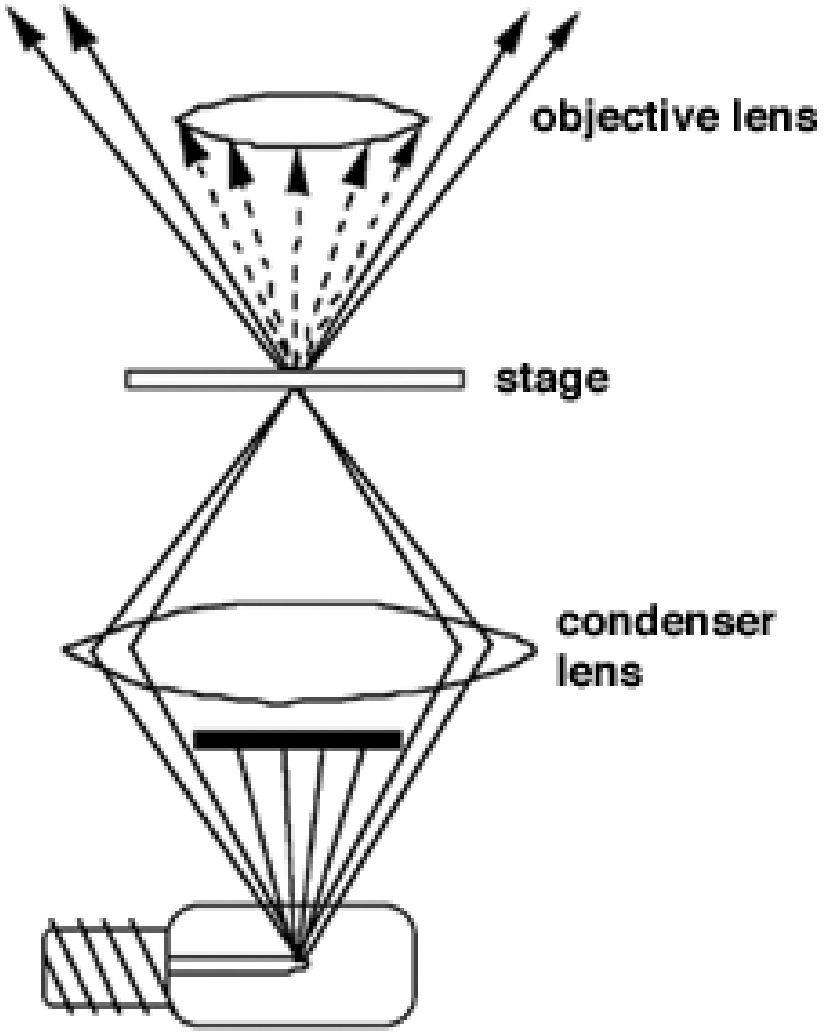
Illumination techniques

- Many techniques are available which modify the light path to generate an **improved contrast image** from a sample.

- Major techniques for generating increased contrast from the sample include **dark field** and **phase contrast.**

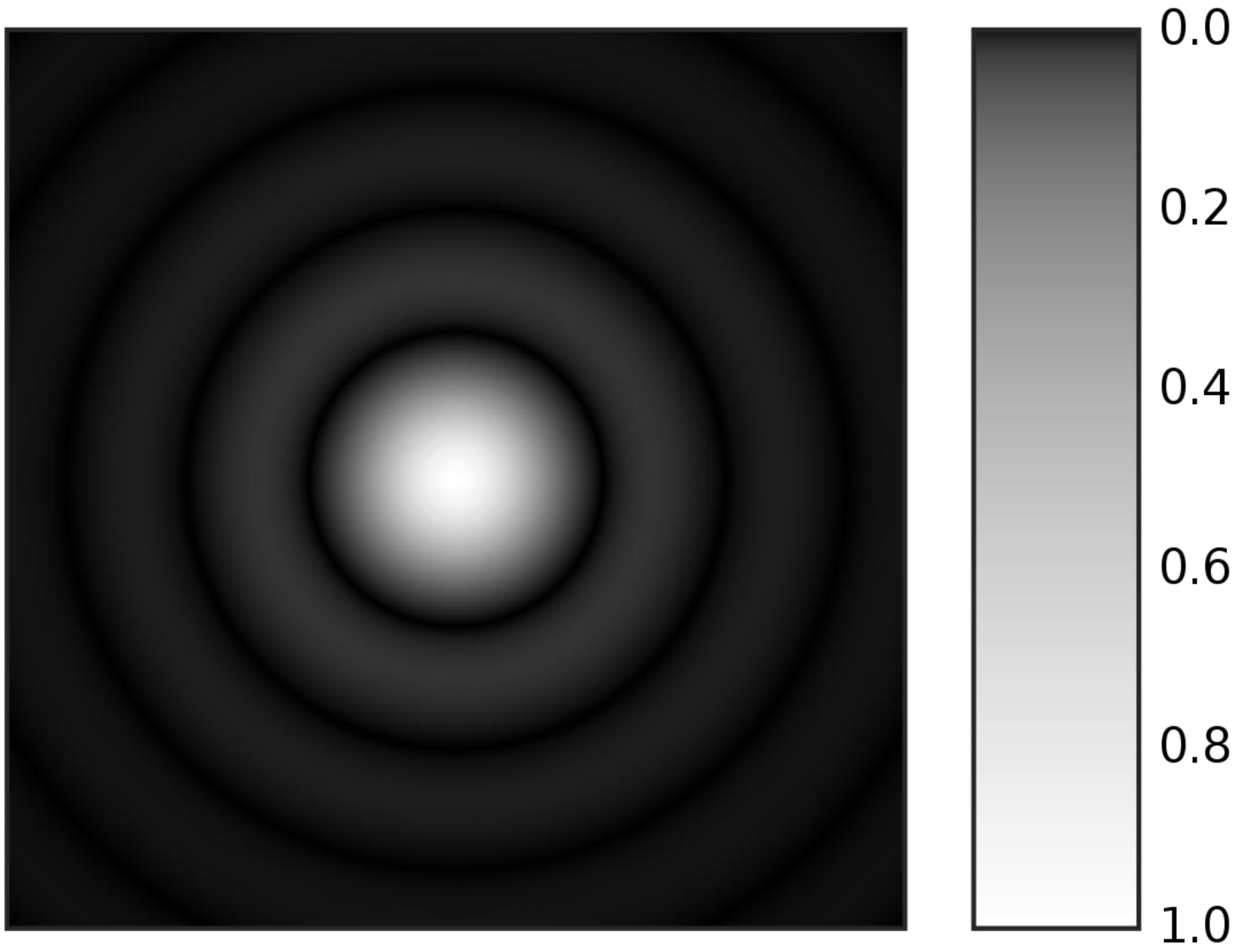
Dark field microscopy

- Dark-field describes an illumination technique used to **enhance the contrast** in **unstained** samples.
- It works by illuminating the sample with **light that will not be collected by the objective lens** and thus will not form part of the image.
- But after hitting the sample, it will enter the objective forming a **bright image** against a **dark background**.


objective lens
stage
condenser
lens

Limitations of light microscopy

- At **very high magnifications** with transmitted light, point objects are seen as **fuzzy discs surrounded by diffraction rings.**
- These are called **Airy disks**.
- Impacts of **diffraction** limit the ability to resolve fine details.


0.0
0.2
0.4
0.6
0.8
1.0

- The **resolving power** of a microscope is taken as the ability to distinguish between two closely spaced points.

- There is therefore a finite limit beyond which it is impossible to resolve separate points in the objective field, known as the **diffraction limit**.

- In practice the lowest value of resolution obtainable with **conventional lenses** is about **200 nm**.

Alternatives

In order to overcome the limitations set by the diffraction limit of visible light, other microscopes have been designed which use **other waves**.

- **Atomic force microscope (AFM)**
- **Scanning electron microscope (SEM)**
- **Scanning ion-conductance microscopy (SICM)**
- **Scanning tunneling microscope (STM)**
- **Transmission electron microscopy (TEM)**
- **Ultraviolet microscope**
- **X-ray microscope**


Thank You!