Light Microscopy

The **optical microscope (light microscope)** is a type of microscope that uses **visible light** and a system of **lenses** to **magnify** images of small objects.

There are two basic types of optical microscopes:

- **simple microscopes**
- **compound microscopes**

- A **simple microscope** is one which uses a **single lens** for magnification, such as a magnifying glass.

- A **compound microscope** uses **several lenses** to enhance the magnification of an object.

- **Modern research microscopes** are **compound microscopes**.

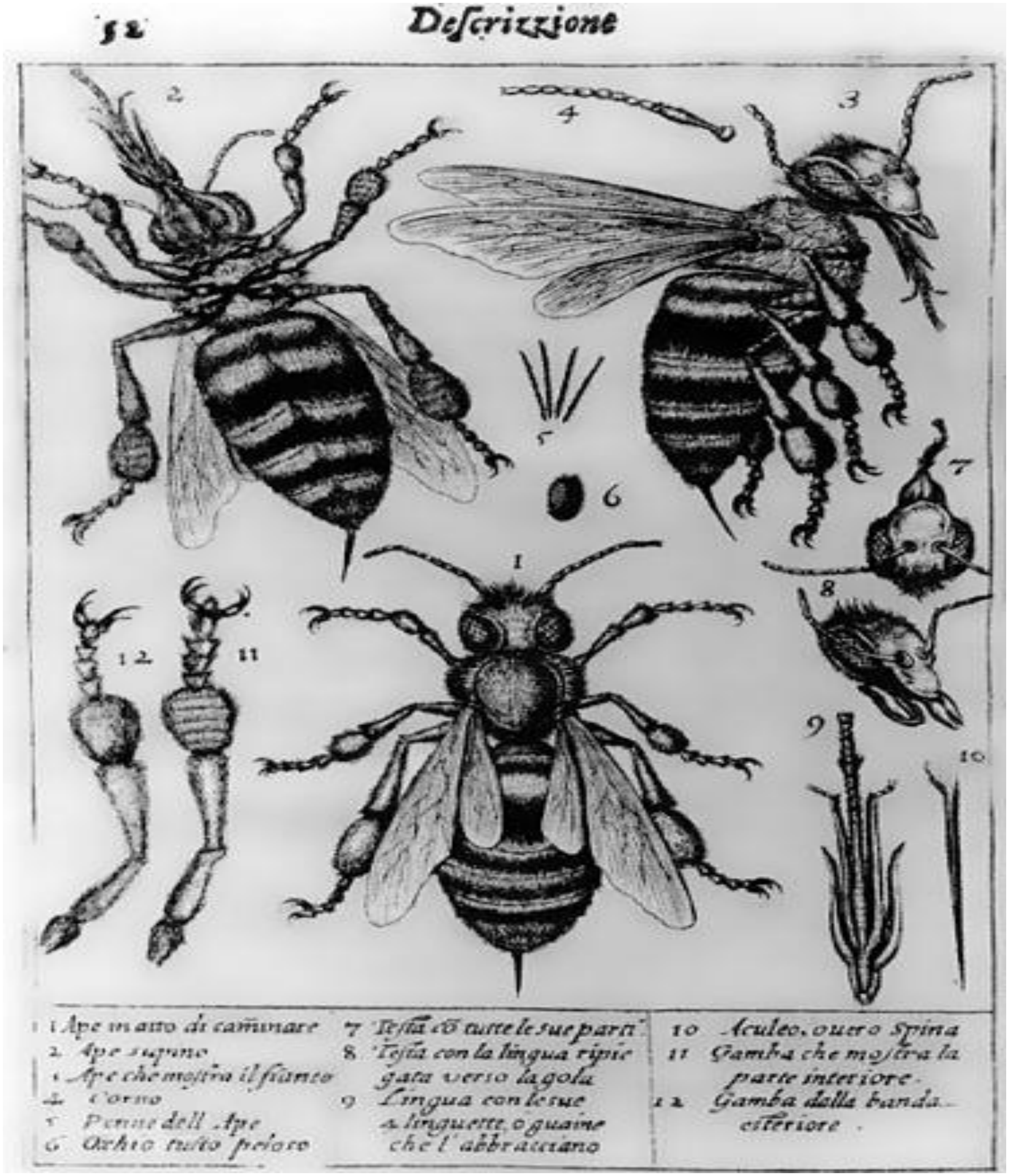
- The **image** from an optical microscope can be captured by **normal, photosensitive cameras** to generate a **micrograph**.

- Originally images were captured by **photographic film**, but modern developments in **CMOS (**complementary metal-oxide semiconductor) and **CCD** (charge-coupled device) **cameras** allow the capture of **digital images**.

- Purely **digital microscopes** are now available which use a CCD camera to examine a sample, showing the resulting image **directly on a computer screen** without the need for eyepieces.

History

- The earliest microscopes were **single lens magnifying glasses** with limited magnification.
- These microscopes date at least as far back as the widespread use of lenses in eyeglasses in the **13th century**.
- The **oldest published image** known to have been made with a microscope is bees by Francesco Stelluti, **1630**.


52
Descrizzione
1 Ape in atto di caminare
2 Ape supino
3 Ape che mostra il fianco
4 Corno
5 Penne dell Ape
6 Occhio tutto peloso
7 Testa cō tutte le sue parti
8 Testa con la lingua ripiegata verso la gola
9 Lingua con le sue 4 linguette, o guaine che l' abbracciano
10 Aculeo, ouero Spina
11 Gamba che mostra la parte interiore.
12 Gamba dalla banda esteriore.

- **Antonie van Leeuwenhoek (1632–1724)** is credited with bringing the microscope to the attention of **biologists**, even though simple magnifying lenses were already being produced in the 16th century.

- Van Leeuwenhoek's home-made microscopes were **simple microscopes**, with a single very small, yet strong lens.

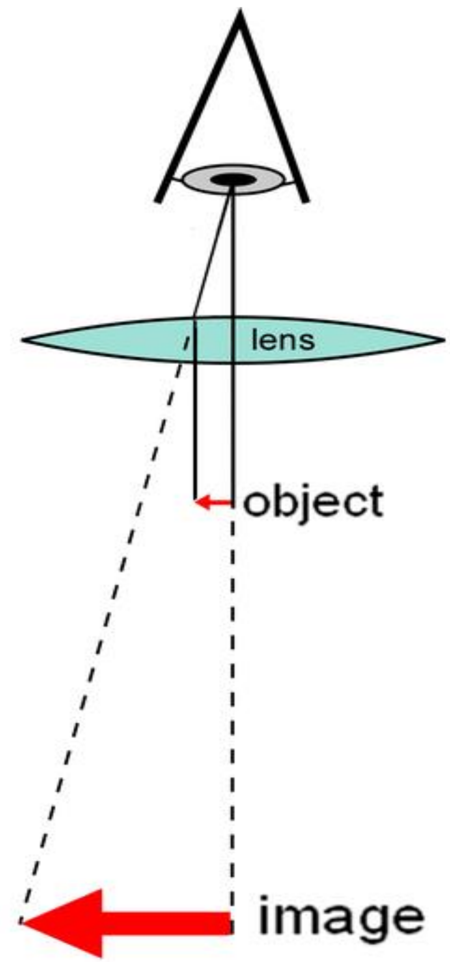
- **Compound microscopes** first appeared in Europe around **1620**.

- Compound microscopes can be **further divided** into a variety of other types of microscopes which differ in their optical configurations, cost, and intended purposes.

Regular microscope

A regular microscope uses a lens or set of lenses to enlarge an object through **angular magnification alone**, giving the viewer an **erect enlarged virtual image.**

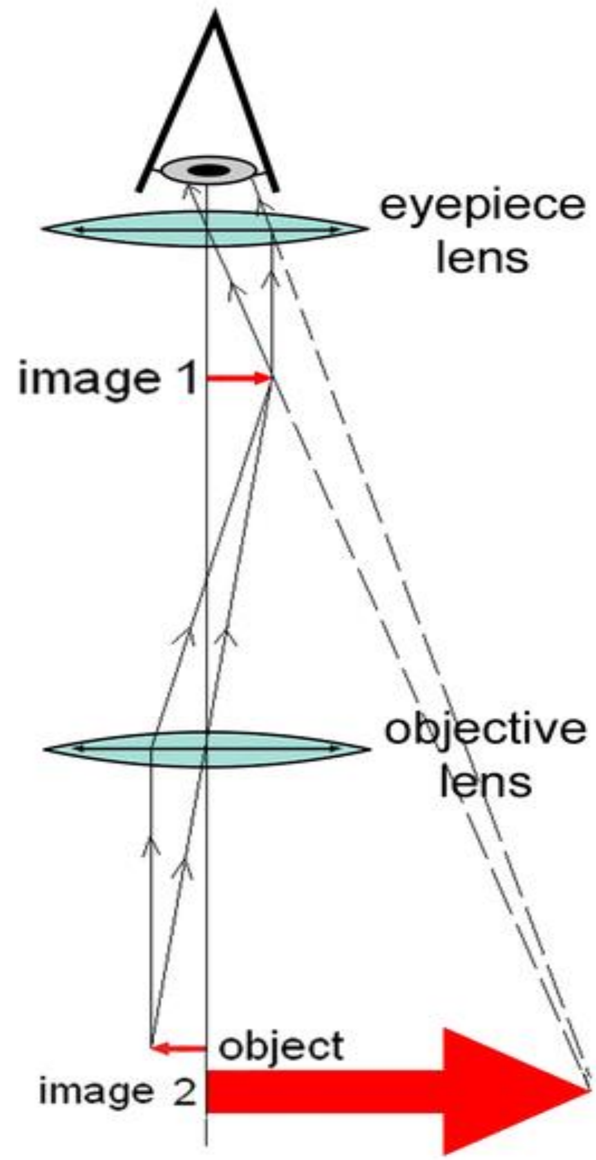
The use of a single convex lens or groups of lenses are found in simple magnification devices such as the magnifying glass.



Compound microscope

A compound microscope uses a lens close to the object being viewed to collect light (called the **objective lens**) which focuses a **real image of the object inside the microscope** (image 1).

That image is then **magnified** by a second lens or group of lenses (called the **eyepiece**) that gives the viewer an **enlarged inverted virtual image of the object** (image 2).



- The use of a **compound objective/eyepiece** combination allows for **much higher magnification**.

- Common compound microscopes often feature **exchangeable objective lenses**, allowing the user to quickly adjust the magnification.

- A compound microscope also enables more **advanced illumination setups**, such as **phase contrast**.

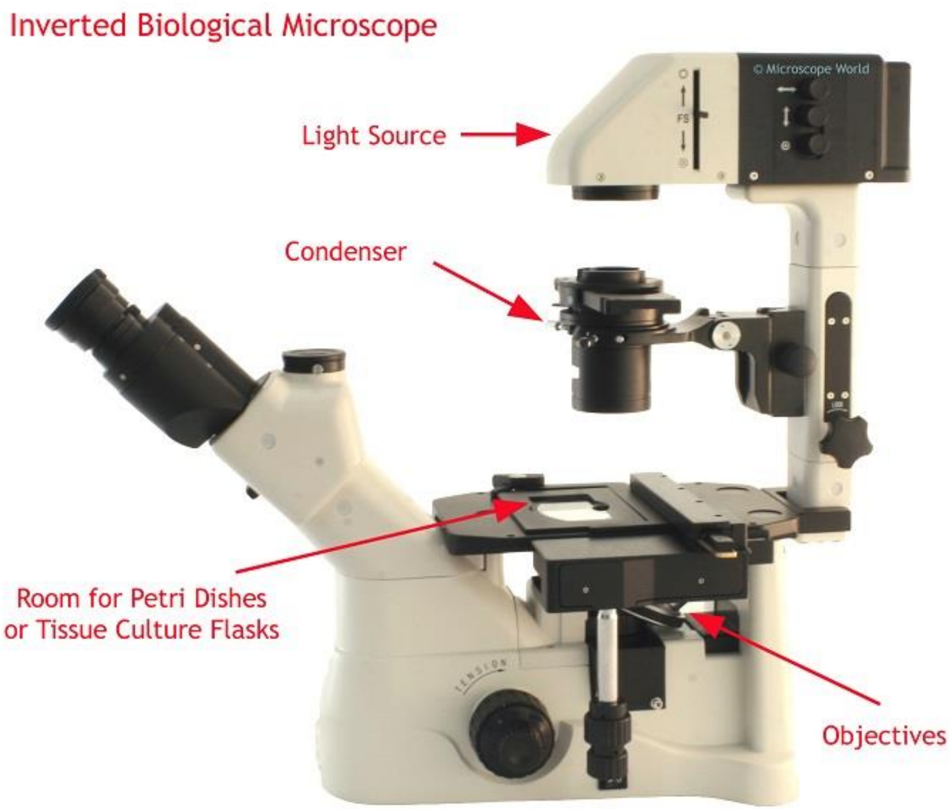
Other microscope variants

There are **many variants** of the compound optical microscope design for **specialized purposes**.

Inverted microscope

It is used for studying samples **from below**.

It is useful for **cell cultures** in liquid, or for **metallography**.

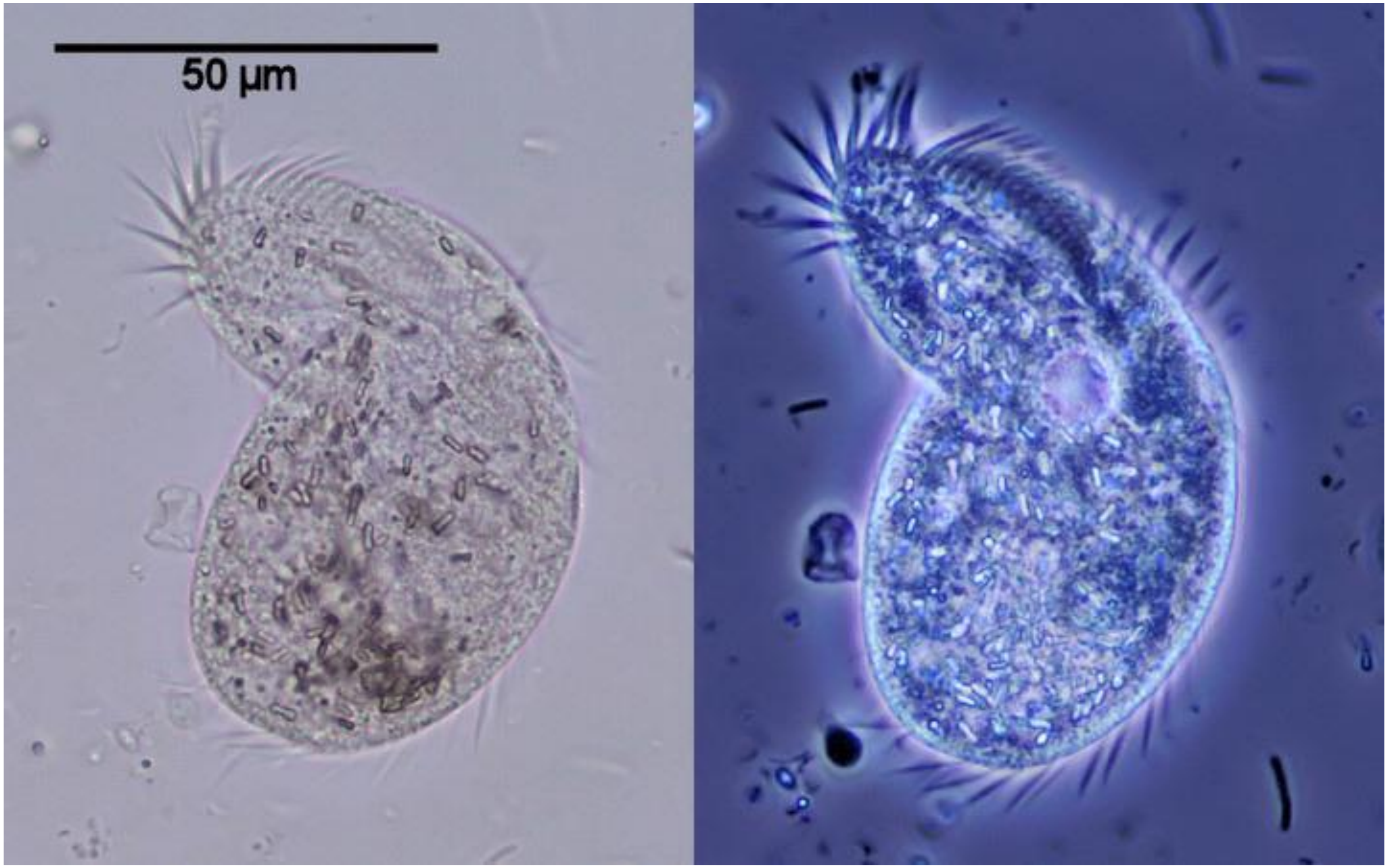


Phase contrast microscope

-- Phase-contrast microscopy is an optical microscopy technique that converts **phase shifts** in light passing through a transparent specimen to **brightness changes** in the image.

-- In other words, it means that light will pass **more slowly through denser material** and therefore the brightness will depend on the density of material.

-- This will result in **various shades of brightness** resulting in an image with **greater details**.


50 µm

Fluorescence microscopy

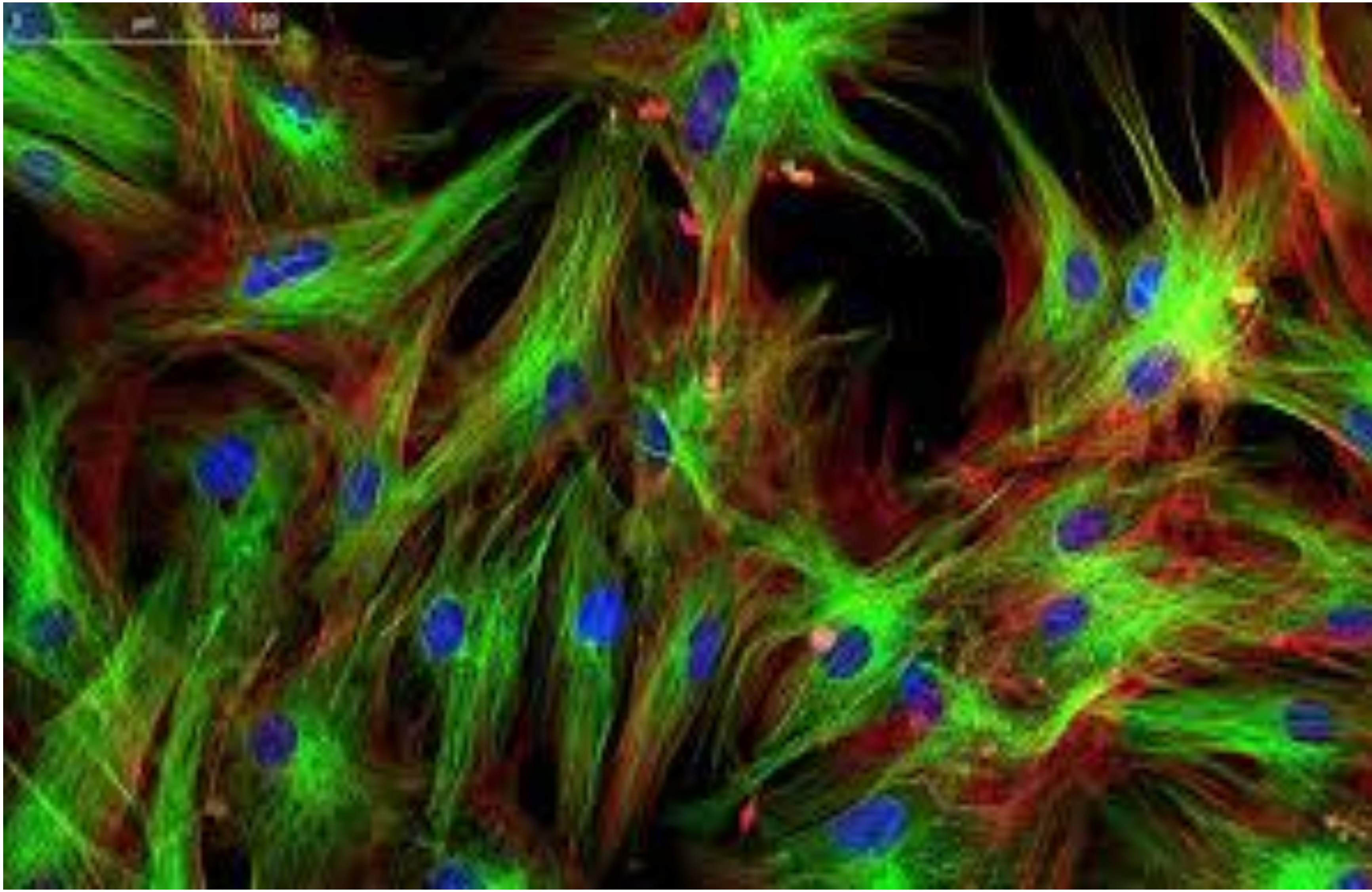
- Modern biological microscopy depends heavily on the development of **fluorescent probes for specific structures** within a cell.
- In contrast to normal **transilluminated** light microscopy, in **fluorescence** microscopy the sample is illuminated through the objective lens with a **narrow set of wavelengths of light**.
- This light **interacts with fluorophores in the sample** which then emit light of a **longer wavelength**.
- It is this **emitted light** which **makes up the image.**

Since the mid 20th century **chemical fluorescent stains**, such as **DAPI** (4′,6-diamidino-2-phenylindole) which binds to DNA, have been used to **label specific structures within the cell.**

More **recent** developments include:

-- **Immunofluorescence**, which uses fluorescently labelled antibodies to recognize specific proteins within a sample.

-- **Fluorescent proteins** like **GFP** (green fluorescent protein) which a live cell can express making it fluorescent.



Confocal microscope

- It uses a **scanning laser** to illuminate a sample for fluorescence.

- It helps in studying **different layers** of a specimen.

Pollen Grain Serial Optical Sections by Confocal Microscopy

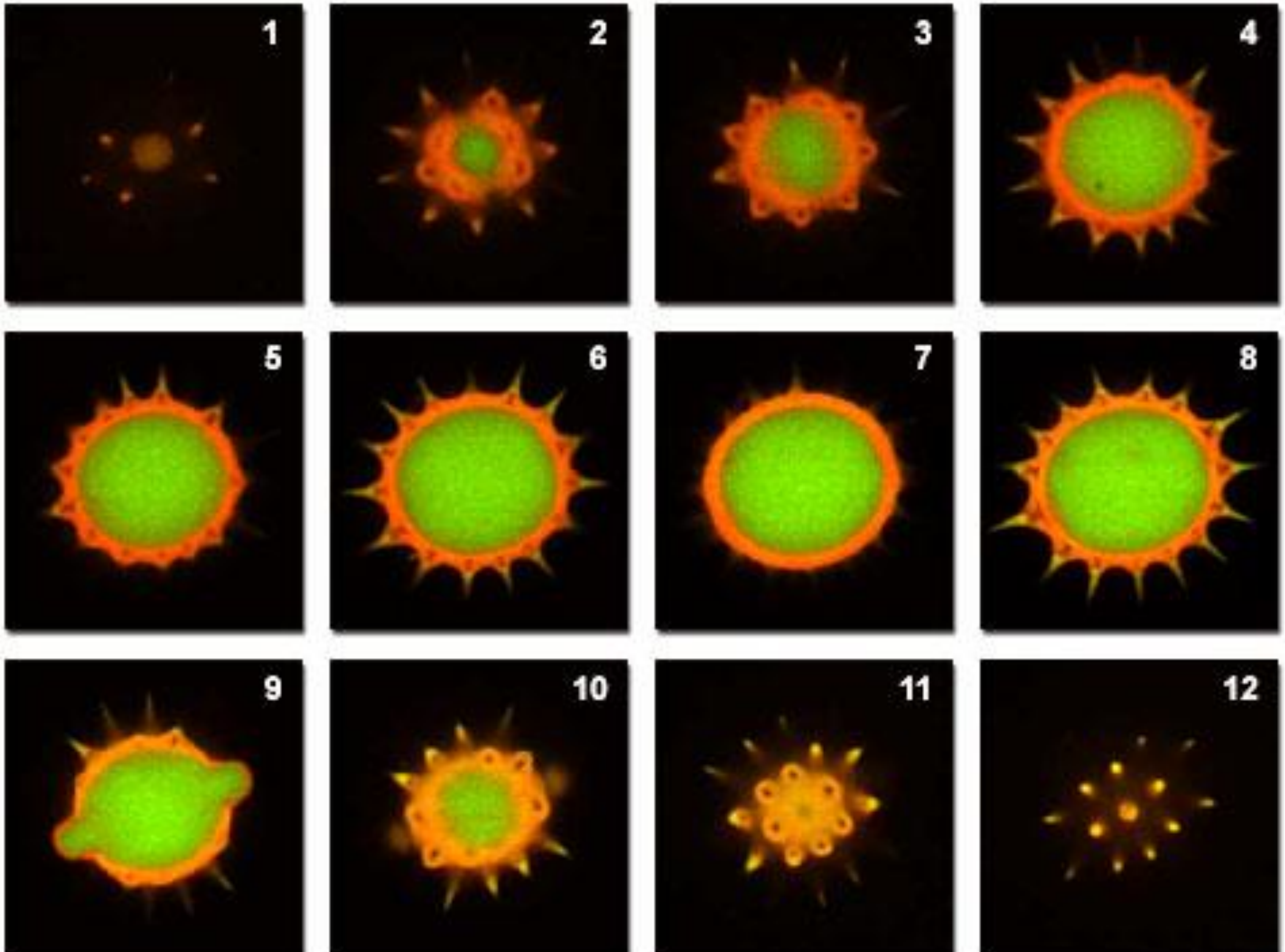


Figure 6

Digital microscope

- A digital microscope is a microscope equipped with a **digital camera** allowing **observation of a sample via a computer**.

- Digital microscopy allows **greater analysis of a microscope image**, for example measurements of distances and areas and quantitation of a fluorescent or histological stain.


Thank You!