High Yield Facts - TOOLS AND TECHNIQUES 1- Microscopy

LIGHT MICROSCOPE

1. **Robert Hook** designed first Microscope
2. **Numerical aperture** is the light gathering capacity of the Microscope.
3. **Working distance** is the distance between the specimen and front lens of the objective.
4. Condenser lens collects light from the light source and focus to the specimen.
5. Objective lens produce the **primary magnified image** of the object and it is **real and inverted.**
6. **Ocular or Eyepiece lens** produces the secondary image of the primary image produced by the objective lens. **It is virtual image**.
7. **Magnification power** of the compound microscope is **2000 – 4000** times and **Resolution** is **0.3 micometer**.
8. **Resolution** of the lens is the property to distinguish two adjacent points in the specimen as two separate regions.
9. A good quality microscope has maximum resolution.
10. **Magnification of compound microscope**

Size of the retinal image seen in the microscope / Size of the retinal image with the normal eye.

11. Maximum resolving power of **human eye is 100 Microns or 0.1 mm**.
12. **Average wave length** of light in the compound microscope is **5850 Angstroms**.
13. **Resolving power** of the compound microscope is **300 Angstroms or 0.3 microns.** It the half wave length of ordinary light.
14. **Oil immersion objective** is used in the compound microscope to get maximum resolution. The space between the cover glass and objective lens is filled with oil like **Cedar Wood Oil** to prevent the loss of light.

ELECTRON MICROSCOPE

1. **Knoll and Ruska** discovered EM in 1932.
2. Source of illumination in EM is electrons.
3. A **Tungston** filament in the Electron Gun is heated using **500 Kv** voltage to emit electrons.
4. **Resolving power** of EM is **5 – 10 Angstroms** and **magnification** is up to **10 Lakhs**.
5. Electromagnetic lenses are used in EM in the place of glass lenses because glass will not pass electrons.
6. Image in the EM is not visible since human eye cannot detect electrons. So a fluorescent screen is used to see the image.
7. Electron microscope may be TEM or SEM or ESEM.
8. Electron microscope will work only in vacuum because air will scatter the electrons.
9. Electron microscope requires dehydrated specimen because water will cause electron scatter.

10. Metals like **Gold and Palladium** are used to impregnate the tissue. These increase the electron scattering power in EM.
11. **Osmium tetroxide** is used as **electron stain** to increase the electron scattering in EM.
12. Scanning Electron Microscope provides the surface details of the specimens.
13. For specimen preparation in SEM, first the tissue is dipped in **liquid Propane** at – 180 degree and dehydrated in alcohol at – 70 degree.
14. **Gold** is coated over the specimen to increase secondary electron emission from the specimen.
15. Secondary electrons from the specimen are used to produce image in SEM.
16. **Magnification power** of SEM is **15000 to 20000 times.**
17. Specimen in EM should be thin (100 Angstrom thickness) because thick specimens will scatter electrons and hence the resolution will be poor.
18. **Enivironmental Scanning Electron Microscope or ESEM** is used to study **wet specimens** like leaf, animal parts etc. It requires no procedures like electron microscope.

PHASE CONTRAST MICROSCOPE

1. **Zernike** in 1940 discovered the phase contrast microscope.
2. Phase contrast microscope is used to study **unstained living specimens** because staining will kill the living organism.
3. Phase contrast microscope has a **Phase plate in the condenser** and an **Anuular diaphragm** in **the ocular lens**.
4. The phase plate and annular diaphragm produces a phase difference in the light and the object appears as bright in the dark background.
5. **No staining** is done in phase contrast microscope.

DARK FIELD MICROSCOPE

1. **Zsigmondy** in 1905 discovered Dark field microscope.
2. It is used to study **unstained living objects**.
3. It has a special condenser which remove light from the center. The object is illuminated by an oblique beam of light.
4. The specimen appears as bright against a dark background.

INTERFERENCE MICROSCOPE

1. Mesten developed Interference microscope.
2. In Interference microscope, light beam is split into two beams. One beam passes through the object and the other besides the object. The two beams are brought together again. It gives the idea about the thickness of the specimen and also the light absorbing property of the specimen.

ULTRA VIOLET MICROSCOPE

1. Ultra violet microscope permits greater resolution and hence greater magnification because the Ultra violet light has a shorter wave length (180 – 400 nm) than the visible light (400 – 800 nm).
2. In Ultra violet microscope, **Quarts or Lithium fluoride** lenses are used since UV rays cannot pass through optical lenses.
3. Image in UV microscope is not visible to human eye and hence a fluorescent screen is used for observation.
4. UV microscope is used for **Quantitative and Qualitative evaluation** of cellular components.
5. Those substances like **DNA and RNA** which absorb in the UV region can be localized even in the living state.

FLUORESCENT MICROSCOPE

1. Some biological materials like **Chlorophyll a, Vitamin A, Riboflavin** etc. emit light when exposed to UV rays. This is called **Autofluorescence.**
2. Non fluorescent substances can be made fluorescent by treating with dyes called Flurochromes like **Acridine Orange, Auramine, Primulin, Fuschin** etc.
3. In fluorescent microscope, **Iodine – Quartz lamp or Mercury vapour lamp** is used to produce UV rays.
4. Fluorescent microscope is used to detect **Chemicals, Microbes, and finding Metabolic pathways.**

X – RAY MICROSCOPE

1. It uses X- ray beam, Electromagnets and Photographic film.
2. Magnification of X-ray microscope is very high similar to that of EM.
3. It is used to study the **3 D structure** of substances in the crystalline state.
4. The technique in X- ray microscope is called **X-ray Crystallography or X- ray diffraction**.
5. **Astbury and Franklin** in 1935 obtain the X- ray diffraction of DNA.
6. It is used with solid crystalline materials.

ULTRA VIOLET SPECTROSCOPY

1. It is used to identify the functional groups and determination of the **molecular structure of an organic compound.**
2. It is specially used to identify compounds **containing multiple bonds**.
3. It is based on the electron transitions of the molecule by the absorption of UV light.
4. The point where maximum UV light absorption takes place is called **Lambda maxima.**