✱ Electron Microscope

✱ Principle → A similar to light microscope except one major difference of using electromagnetic lens, rather than optical lens to focus a high velocity electron beam instead of visible light.

→ A beam of electrons are used.

✱ Due to short wavelength of electron the resolving power of electron microscope is very high.

✱ Not used to study live cells. It is used to study dead cells.

✱ It is of two types:

- Transmission electron microscope (TEM)
- Scanning electron microscope (SEM)

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* Transmission electron microscope (TEM)

→ Most commonly used electron microscope.

→ Called as transmission electron microscope because it forms an image from electrons that are transmitted through the specimen being examined.

* Scanning electron microscope (SEM)

→ Produces image from electrons deviated from a specimen's outer surface.

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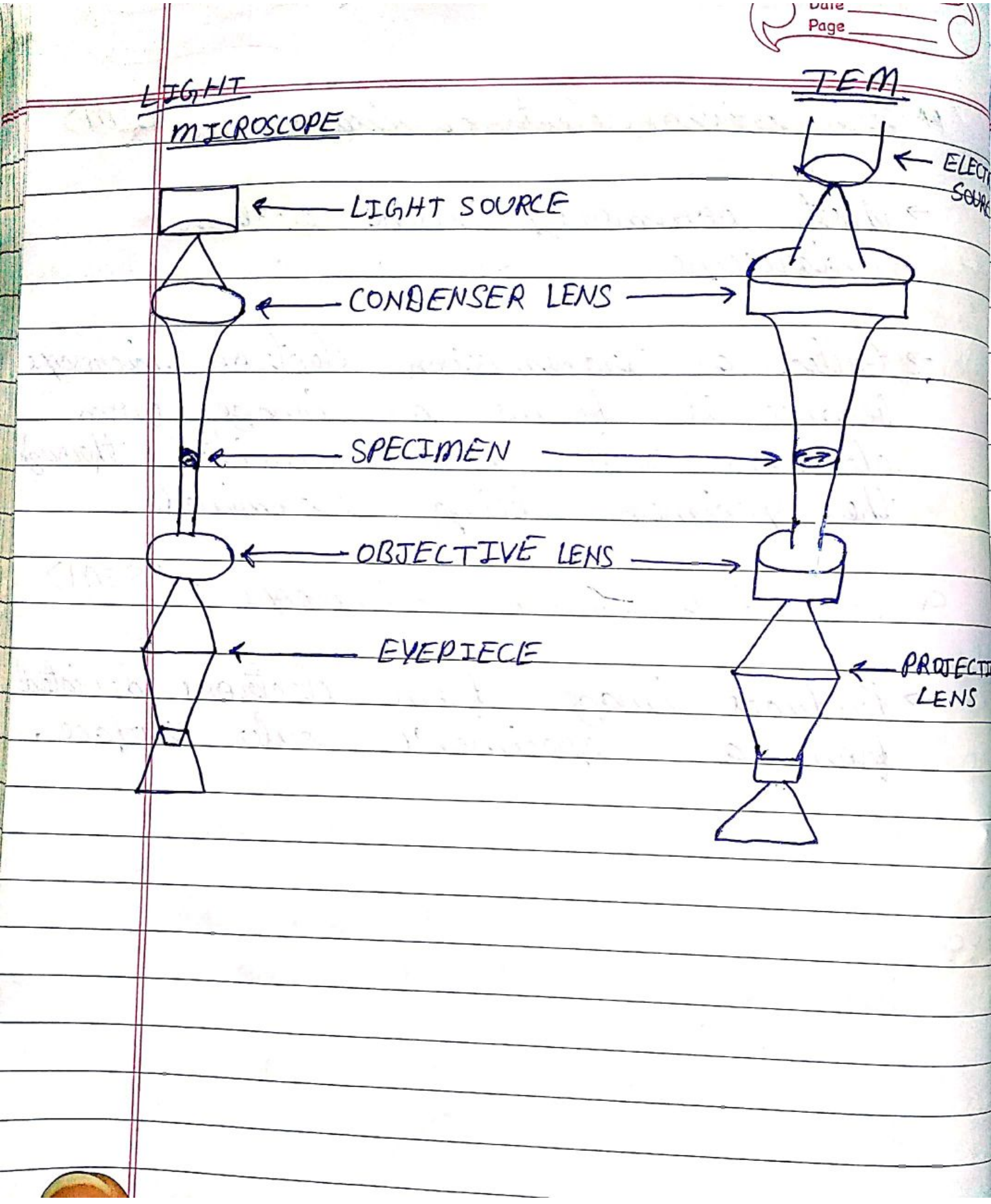

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* Difference between Light and Electron Microscope

Features	Light microscope	Electron microscope
1. Highest practical magnification	1000-1500	over 1 lakh
2. Best resolution	0.2 µm	0.5nm
3. Radiation Source	Visible light	Electron beam
4. Medium of Travel	Air	High vaccum
5. Type of Lens	Optical lens/glass	Electro-magnetic lens
6. Specimen mount	Glass slide	Metal grid (usually copper).

* Transmission Electron Microscope (TEM)

→ In transmission electron microscope, a condenser lens focuses the electron beam on to the specimen and electrons are transmitted through the specimen.

→ Portion of beam absorbed by specimen is minimum.

→ To be absorbed an electron must loose all its energy to the specimen.

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→ Image formation in the electron microscope depends on differential scattering of electrons by parts of the specimen.

→ Consider a beam of electrons focused on the screen. If no specimen is there present in the column, the screen would be evenly illuminated by the beam of

electrons producing an image that is uniformly bright.

→ While a specimen is placed in the path of the beam, some of the electrons strike the specimen and are scattered away.

→ Electrons - come in contact with sample

- Elasticity → either scattered that is without loss of energy
- Inelasticity → that is transferring some of the energy to the atom (loss of some electrons to the atom).

→ Interaction between the incoming fast electron and atomic nucleus give rise to elastic scattering.

→ Interaction between fast electron and atomic electron result in inelastic scattering.

→ Some of the electrons passing through the specimen are scattered and the reminder is focused to form an image (same as in light microscope).

→ Image can be observed on a phosphorescent screen or recorded either on the photographic plate or with a high resolution digital camera.

→ Because of scattering, electrons are lost from the beam, the dense specimen show up in the image as area of reduced electron flux which look dark.

→ Scattering of electron contribute to the contrast because the electrons are absorbed by atoms in the

air. The entire tube between electron source and detector is maintained under an ultra-high vaccum.

* Amount of scattering for any particular specimen depends on

i) its density

ii) overall thickness

iii) relatively independent on atomic number

iv) chemical composition

v) and other specimen properties.

* Specimen prepared for transmission electron microscope has to be :-

i) fairly uniform thickness - therefore almost no contrast arise from thickness.

ii) atomic number also remains almost constant - so overall contrast is very low and the specimen appears featureless in TEM.

* To produce scattering contrast - the sample is generally stained with electron dense material So before or after slicing, the specimen is immersed in a solution that contain salts and heavy metal such as uranium and lead.

* Degree of impregnation or staining with these cellular constituents vi various degree of contrast.

* Darker area in the image are where few electrons are transmitte through the sample due to thickness or staining.

Scanning Electron Microscope (SEM)

i) View the surface of specimen.

ii) Sample is fixed, dried and coated with thin layer of heavy metal such as gold or a mixture of gold and palladium.

iii) Specimen is than scanned with a very narrow beam of electron.

iv) Molecules in the specimen are excited and they release secondary electrons that are captured by a detector and generates an image of specimen's surface.

v) Contrast arises when different part of specimen generate differing amount of secondary electrons as the electron's beam strikes them.

vi) Areas which generate a large number of secondary electrons

will appear brighter than areas that generate fewer secondary electrons.

vii) The resolving power of SEM is limited by the thickness of of metal coating than that of TEM.

viii) The image produced appear 3-dimensional in SEM whereas TEM produces 2-dimensional image