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Question

- 1) Discuss the procedure involve in making of glass knife for cutting in ultrathin sections
- 2) Draw a knife maker and label it

ANSWERS

1- The procedure is called **Ultramicrotomy** is a method for cutting specimens into extremely thin slices, called ultra-thin sections, that can be studied and documented at different magnifications in a transmission electron microscope (TEM). It is used mostly for biological specimens, but sections of plastics and soft metals can also be prepared. Sections must be very thin because the 50 to 125 kV electrons of the standard

electron microscope cannot pass through biological material much thicker than 150 nm. For best resolutions, sections should be from 30 to 60 nm. This is roughly the equivalent to splitting a 0.1 mm-thick human hair into 2,000 slices along its diameter, or cutting a single red blood cell into 100 slices.[1]

A **microtome** (from the Greek *mikros*, meaning "small", and *temnein*, meaning "to cut") is a tool used to cut extremely thin slices of material, known as sections. Important in science, microtomes are used in microscopy, allowing for the preparation of samples for observation under transmitted light or electron radiation.

Microtomes use steel, glass, or diamond blades depending upon the specimen being sliced and the desired thickness of the sections being cut. Steel blades are used to prepare sections of animal or plant tissues for light microscopy histology. Glass knives are used to slice sections for light microscopy and to slice very thin sections for electron microscopy. Industrial grade diamond knives are used to slice hard materials such as bone, teeth and plant matter for both light microscopy and for electron microscopy. Gem quality diamond knives are used for slicing thin sections for electron microscopy.

Ultra-thin sections of specimens are cut using a specialized instrument called an "ultramicrotome". The ultramicrotome is fitted with either a

diamond knife, for most biological ultra-thin sectioning, or a glass knife, often used for initial cuts. There are numerous other pieces of equipment involved in the ultramicrotomy process. Before selecting an area of the specimen block to be ultra-thin sectioned, the technician examines semi thin or "thick" sections range from 0.5 to 2 μm . These thick sections are also known as *survey sections* and are viewed under a light microscope to determine whether the right area of the specimen is in a position for thin sectioning. "Ultra-thin" sections from 50 to 100 nm thick are able to be viewed in the TEM.

Tissue sections obtained by ultramicrotomy are compressed by the cutting force of the knife. In addition, interference microscopy of the cut surface of the blocks reveals that the sections are often not flat. With Epon or Vestopal as embedding medium the ridges and valleys usually do not exceed 0.5 μm in height, i.e., 5–10 times the thickness of ordinary sections (John J. Bozzola).

A small sample is taken from the specimen to be investigated. Specimens may be from biological matter, like animal or plant tissue, or from inorganic material such as rock, metal, magnetic tape, plastic, film, etc. The sample block is first trimmed to create a block face 1 mm by 1 mm in size. "Thick" sections (1 μm) are taken to be looked at on an optical microscope. An area is chosen to be sectioned for TEM and the

block face is re-trimmed to a size no larger than 0.7 mm on a side. Block faces usually have a square, trapezoidal, rectangular, or triangular shape. Finally, thin sections are cut with a glass or diamond knife using an ultramicrotome and the sections are left floating on water that is held in a boat or trough. The sections are then retrieved from the water surface and mounted on a copper, nickel, gold, or other metal grid. Ideal section thickness for transmission electron microscopy with accelerating voltages between 50kV and 120kV is about 30–100 nm.

HISTORY

One of the first devices for the preparation of such cuts was invented in 1770 by George Adams, Jr. (1750–1795) and further developed by Alexander Cummings.[Quekett, John (1848)].

The device was hand operated, and the sample held in a cylinder and sections created from the top of the sample using a hand crank.(Hill, John (1770)

In 1835, Andrew Prichard developed a table based model which allowed for the vibration to be isolated by affixing the device to the table, separating the operator from the knife.[Gilbert Morgan Smith]

Occasionally, attribution for the invention of the microtome is given to the anatomist Wilhelm His, Sr. (1865), In his *Beschreibung eines Mikrotoms* (German for *Description of a Microtome*), Wilhelm wrote:

The apparatus has enabled a precision in work by which I can achieve sections that by hand I cannot possibly create.

Namely it has enabled the possibility of achieving unbroken sections of objects in the course of research.

Other sources further attribute the development to a Czech physiologist Jan Evangelista Purkyně. Several sources describe the Purkyne model as the first in practical use.[[Werner Gerabek, Bernhard](#)]

The obscurities in the origins of the microtome are due to the fact that the first microtomes were simply cutting apparatuses, and the developmental phase of early devices is widely undocumented.

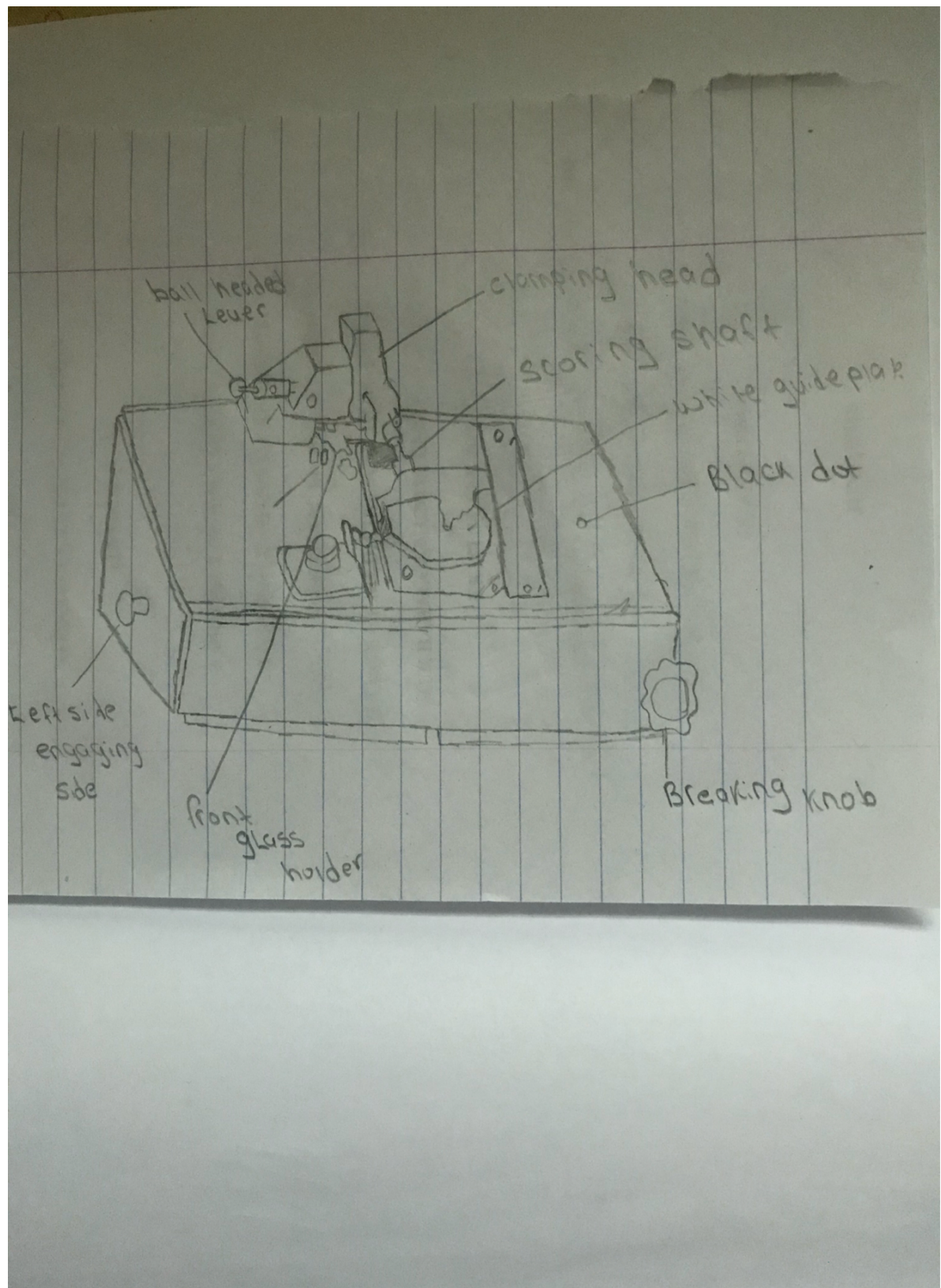
At the end of the 1800s, the development of very thin and consistently thin samples by microtomy, together with the selective staining of important cell components or molecules allowed for the visualisation of microscope details.

Today, the majority of microtomes are a knife-block design with a changeable knife, a specimen holder and an advancement mechanism. In most devices the cutting of the sample begins by moving the sample over

the knife, where the advancement mechanism automatically moves forward such that the next cut for a chosen thickness can be made. The section thickness is controlled by an adjustment mechanism, allowing for precise control.

QUESTION 2

Diagram of a knife maker



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