9. 6 Biotechnology

In life science applications, photomultiplier tubes are mainly used for detection of fluorescence and scattered light. Major equipment used for life science includes cell sorters, fluorometers and DNA sequencers.

9. 6. 1 Overview

(1) Cell sorters

When light is irradiated onto a rapidly flowing solution which contains cells or chromosomes, a scattered light or fluorescence is released from the cells or chromosomes. By analyzing this scattered light or fluorescence, it is possible to elucidate cell properties and structures and separate the cells based on these properties. This field is known as flow cytometry. A cell sorter like the one illustrated in Figure 9-26 is most frequently used. The cell sorter is an instrument that selects and collects only specific cells labeled by a fluorescent substance from a mixture of cells in a solution.

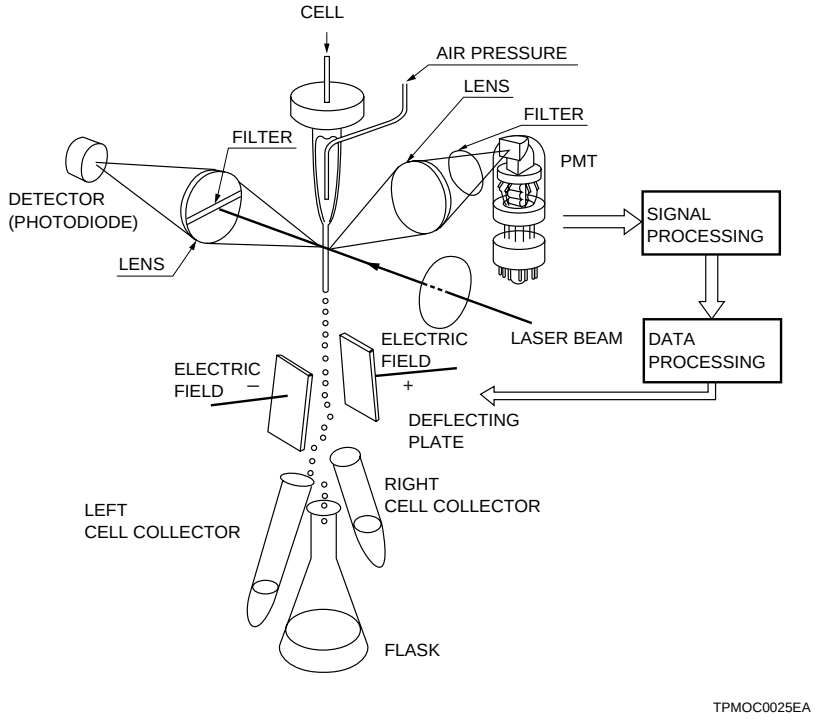

Figure 9-26: Major components for a cell sorter

In a cell sorter, a fluorescent probe is first attached to the cells. The cells pass through a thin tube at a fixed velocity. When each cell passes through a small area onto which an intense laser beam is focused, the fluorescence is emitted from the cell and is detected by a photomultiplier tube. The photomultiplier tube outputs an electrical signal in proportion to the number of fluorescent molecules attached to each cell. At the same time, the laser beam light is scattered forward by the cell, and detecting this scattered light gives information on the cell volume. After processing these two signals, the cell sorter creates an electrical pulse that deflects a drop of liquid, containing the desired cell into one of the collection tubes.

(2) Fluorometers

While the ultimate purpose of the cell sorter explained above is to separate cells, the fluorometer[16] is used to analyze cells and chemical substances by measuring the fluorescence or scattered light from a cell or chromosome with regard to such factors as the fluorescence spectrum, fluorescence quantum efficiency, fluorescence anisotropy (polarization) and fluorescence lifetime. (See Figure 9-27.)

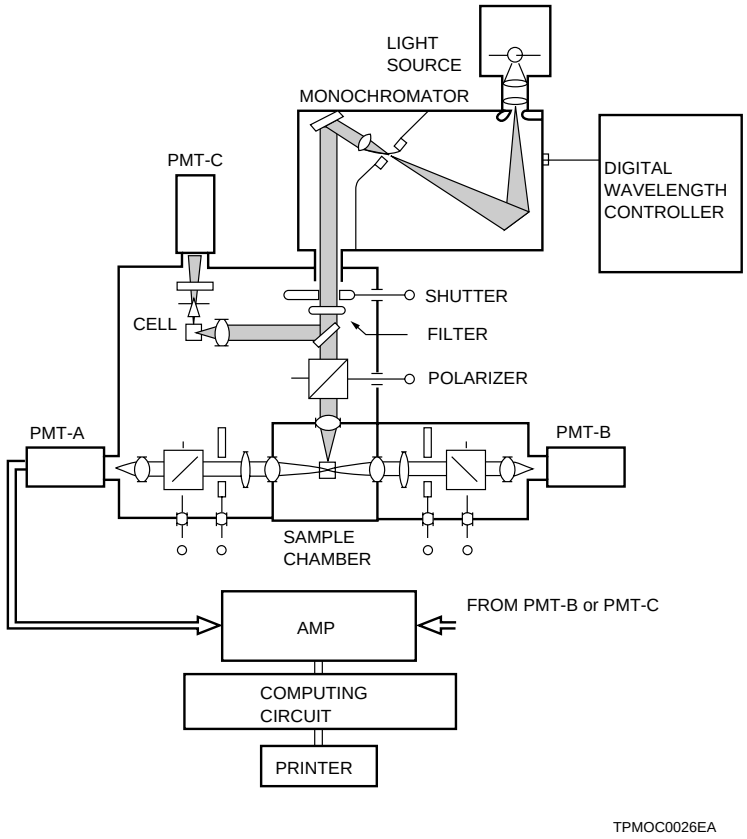

Figure 9-27: Automatic fluorescence-depolarization photometer

The basic configuration of the fluorometer is nearly identical with that of the fluorospectrophotometer and thus a description is omitted here. There are a variety of models of fluorometers which are roughly categorized into: filtering fluorescence photometers, spectrofluorescence photometers, compensated-spectrofluorescence photometers, fluoroanisotropy analyzers, and phase fluorescence lifetime measurement systems. Of these, the fluoroanisotropy analyzer is an instrument specially dedicated to measurement of fluorescence-depolarization.

When performing research on biological samples such as proteins, nucleic acid and lipid membranes, rotational relaxation of a fluorescent molecule takes place only slowly and the fluorescence is polarized in most cases. It is still necessary to compensate for the effect of fluorescence depolarization when measuring quantum efficiency and spectrum. For this purpose, the automatic fluorescence-depolarization photometer uses a pair of photomultiplier tubes which detect the two polarized components at the same time.

(3) DNA sequencers

This is an instrument used to decode the base arrangement of DNA extracted from a cell. The principle of a DNA sequencer is shown in Figure 9-28. An extracted DNA segment is injected onto gel electrophoresis plate along with a fluorescent label which combines with the DNA. When an electric potential is applied across the gel, the DNA begins to migrate and separate based on size and charge. When the DNA segment reaches the position of the scanning line, it is excited by a laser, causing only the portion with the labeling pigment to give off fluorescence. This fluorescent light is passed through monochromatic filters and detected by photomultiplier tubes. Computer-processing of the position at which the fluorescence has occurred gives information on where the specific bases are located. The DNA sequencer is used for the genetic study of living organisms, research into the cause and treatment of genetic diseases and decoding of human genes.

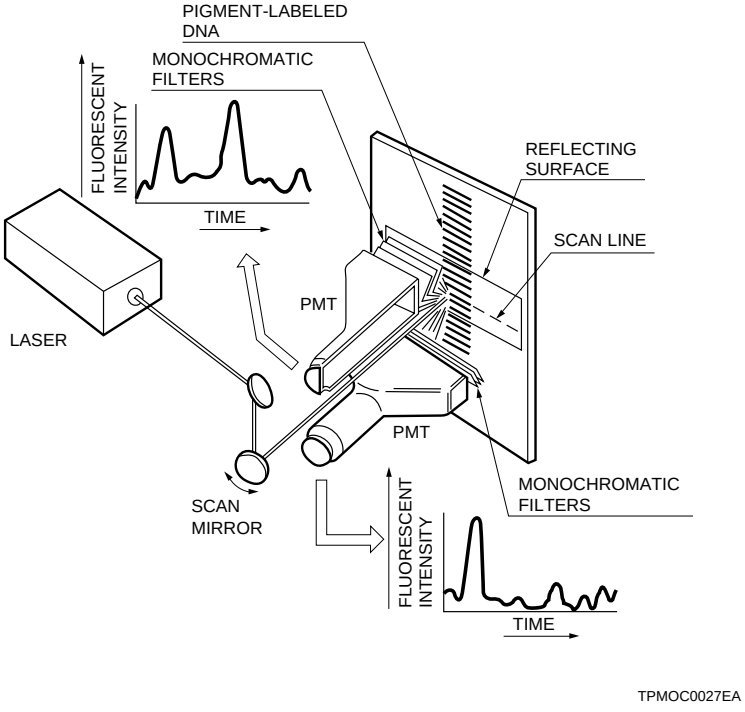

Figure 9-28 Principle of a DNA sequencer

9. 6. 2 Major characteristics required of photomultiplier tubes

Because the photomultiplier tube detects very-low fluorescence emitted from a cell or DNA, the following characteristics are required as in the case of spectrophotometry.

a) High stability

b) Low dark current

c) High signal-to-noise ratio

d) Wide spectral response

e) Low hysteresis

f) Excellent polarization properties