Cells with similar physical and chemical properties form a cluster on the graph. This graph is called a scattergram. The automated haematology analysers (XE Family®) of Sysmex are also based on the principle of flow cytometry [1].
**Fluorescence microscopes and confocal laser scanning microscopes**

A fluorescence microscope offers features that the cells stained with a fluorescent dye are exposed to light to induce excitation\[^{10}\] and the entire view of the cells is observed. A confocal laser scanning microscope, on the other hand, observes sectional images of the cells after excitation with laser. The focal plane within the cell is scanned with a laser\[^{11}\], and the spatial distribution\[^{12}\] of fluorescence and reflected light on the focal plane is recorded. These records are processed with a computer to yield sectional images. The images taken with a confocal laser scanning microscope have high spatial resolution, allowing localization\[^{13}\] of the target substance stained with fluorescence. It is also possible to analyse the three-dimensional structure of cells by overlapping multiple sectional images of different focal planes within the cells.

**Fluorescence microscope images compared with confocal laser scanning microscope images**

Images above show the protein expressed on cell surface observed with a fluorescence microscope and a confocal laser scanning microscope after staining with a fluorescent dye. The fluorescence microscope can capture fluorescence from the entire cell. The confocal laser scanning microscope provides the sectional images of the cells with high-resolution localisation of the target substance. Precise information about cells can be obtained by comparing and analysing the images taken with a fluorescence microscope and a confocal laser scanning microscope.

**What is an electron microscope?**

Electron microscopes are the only means available for direct observation of the activity of viable cells at a nano-scale\[^{14}\]. Highly magnified images with high resolution\[^{15}\] can be obtained by the use of electron beams\[^{16}\] with a wavelength of 0.01 nm, which is shorter than that of visible rays\[^{17}\] (ca. 350–800 nm). As compared to biological microscopes which utilize visible rays and yield images with a resolution of 200–300 nm, electron microscopes using electron beams yield images with remarkably higher resolution of 0.35 nm (in case of 150 kV transmission electron microscopes).

The electron microscopes, which were conventionally used for observation of ultramicrostructure, have recently begun to be used also to perform qualitative analysis\[^{18}\] and quantitative analysis\[^{19}\] of elements constituting samples by the use of characteristic X-ray (specific to elements and released from the samples in response to application of an electron beam). Two-dimensional distribution of elements can be visualized by overlapping an X-ray map on an electron microscope picture. These techniques related to electron microscopy have been applied not only to studies on biosamples but also to analysis of various materials frequently.
Comparison of images obtained with two types of electron microscope

**Scanning electron microscope**
Images are obtained by analysing the electron beams reflected on the cells dried by specific methods. The detailed surface structure of cells can be observed using this type of electron microscope.

**Transmission electron microscope**
Electron beams are applied to the cells cut into thin slices. The beams passing through the cell slices are analysed to yield images. The detailed inner structure of cells can be observed using this type of electron microscope.

*courtesy: Hitachi High-Technologies Corporation*
Terminology

1 Bioimaging technology
The targets in cells or tissues are marked with various dyes, fluorescent dye or colloidal gold (applicable to electron microscopes), to visualize the location and kinetics of the target.

2 Flow cytometer
Small particles such as cells are dispersed in a fluid, and the fluid is flowed through a small nozzle for optical analysis of individual particles.

3 Fluorescent dyes
A collective term for substances which, after absorbing electromagnetic radiation such as light, themselves emit radiation, usually of a longer wavelength than that of the absorbed radiation (e.g. absorbing ultraviolet light and emitting visible light). If a fluorescent dye is bound to particles or substances, it allows accurate location, observation and measurement of potential changes in the target.

4 Buffer
Solutions composed, for example, of a mixture of weak acid and base, so that the pH may remain stable at about the neutral level. Cells manipulated in a buffer allow to perform experiments while avoiding the influence from acid or base.

5 Laser
Light amplified into coherent radiation (utilized for flow cytometers and confocal laser scanning microscopes).

6 Forward scatter
Laser (635 nm) scattered in the forward direction when applied to the cells flowing through a flow cell. Serves as an indicator of cell size.

7 Side scatter
Laser (635 nm) scattered in the 90-degree (side) direction when applied to the cells flowing through a flow cell. Serves as an indicator of complexity inside cells (nuclear shape and size, density of organelles).

8 Side fluorescence
Fluorescence emitted in the right-angle direction from the cells (stained with a fluorescent dye) flowing through a flow cell due to excitation by the laser applied. Serves as an indicator of the intensity of staining of the cells with the fluorescent dye.

9 XE Family
A series of products of Sysmex represented by automated haematology analyser XE-5000 and XE-2100. XT-2000i, XT-1800i, XS-1000i and XS-800i are also available in this series.

10 Excitation
Substances in stable status acquire high energy under the influence from outer factors such as light, heat, electricity, magnetism, etc. When fluorescent dyes are excited, energy is produced in the form of light (fluorescence).

11 Scanned with a laser
A technique which converts images into electric signals. Scanning is used for example for facsimiles which convert the two-dimensional information into a single strand of information (one-dimensional information) by tracing the information on each line from the left to right and from top to bottom. A confocal laser scanning microscope converts three-dimensional information into two-dimensional information.

12 Spatial distribution
In this context, it means three-dimensional distribution.

13 Localisation
The location at which a substance is present.

14 Nano-scale
Equal to nm (nanometer), equivalent to a millionth of 1 mm. Used for expressing the size of organelles (e.g. ribosome 200 nm), viruses (e.g. T4-phage 100 nm) and so on.

15 Resolution
The minimum discernable distance between two points. It is about 350 nm for light microscopes and about 0.35 nm for electron microscopes.

16 Electron beam
Flows of electrons seen on the cathode plates and during electric discharge.

17 Visible ray
Electromagnetic waves of wavelengths visible for humans.

18 Qualitative analysis
Analysis to identify the components of a given sample.

19 Quantitative analysis
Determination of the amount of specific components of a given sample.
References

