

Principle for automated leukocyte differentiation with XE Family analysers, making use of bioimaging technology

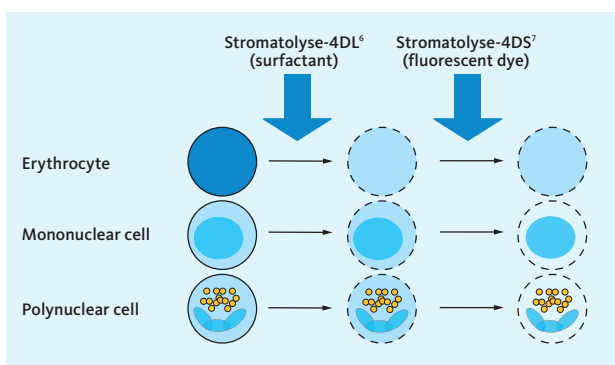
The Cell Analysis Center – Scientific Bulletin Part 4

The XE family is capable of automated leukocyte differentiation by which blood cells are sensitized to surfactant¹ and fluorescent dye². Morphological and physical/chemical properties of various types of cells are taken into account, and they are analysed under the principle of flow cytometry³ [1]–[6]. Following recent remarkable advances in bioimaging technology, it is now possible to observe the activity of organisms at the cell level instantaneously, with ultrahigh sensitivity CCD cameras and ultrahigh resolution electron microscopes. Making use of such rapidly advancing bioimaging technology⁴, Sysmex has developed the XE Family analysers capable of clarifying the morphological characteristics of leukocytes flowing through the flow cell⁵. This bulletin visually illustrates the principle of measurement with these devices.

Principle for automated leukocyte differentiation [1]–[6]

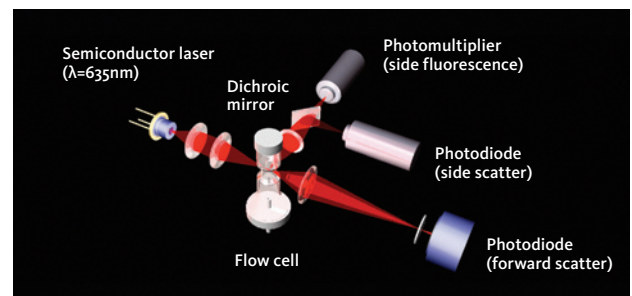
Pretreatment with a special reagent

Stromatolyser-4DL (a surfactant), which is a reagent specific to the XE Family, induces haemolysis of erythrocytes and the formation of ultramicroscopic pore in the leukocyte cell membrane. Stromatolyser-4DS (a fluorescent dye) is introduced through the ultramicroscopic pores into the cells to stain nucleic acids⁸ and organelles⁹ [7], [8].

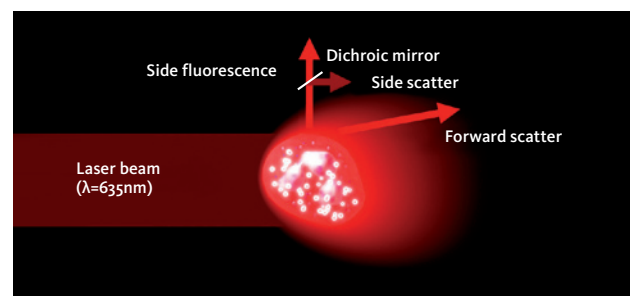


Detection under the principle of flow cytometry

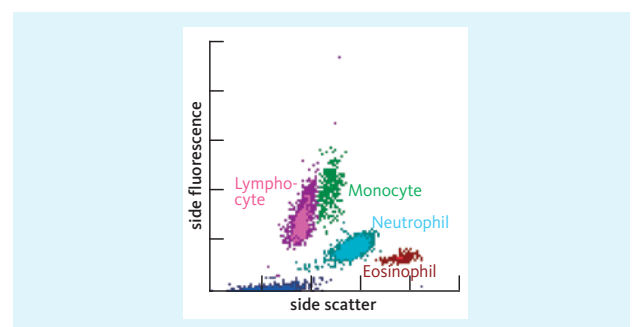
The leukocytes sensitized to the two special reagents are detected by the principle of flow cytometry. The signals from the cells related to side scatter¹⁰ (an indicator of complexity inside the cells) and side fluorescence¹¹ (an indicator of staining intensities of the cells) are depicted on a scattergram¹². Leukocytes¹³ with similar physical and chemical properties form a cluster¹⁴. The clusters formed are used to differentiate leukocytes.



Principle of flow cytometer



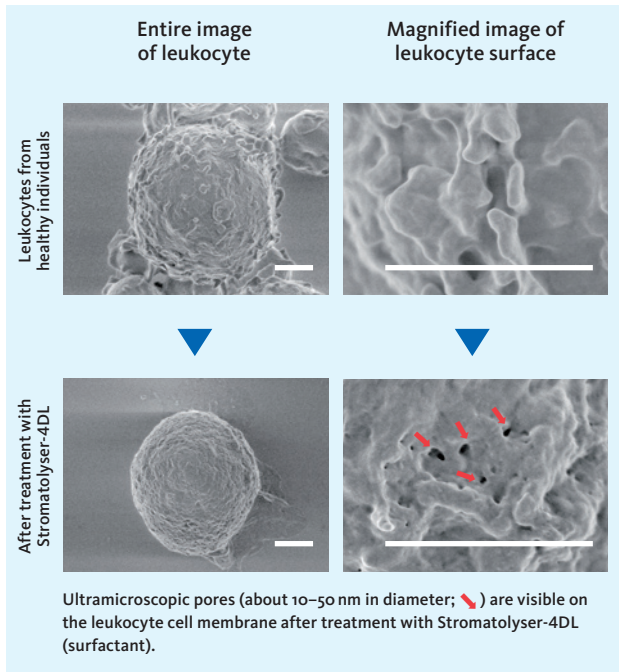
Light detected from cells



XE Family WBC differential scattergram

Changes in ultrastructure following exposure to Stromatolyser-4DL (surfactant)

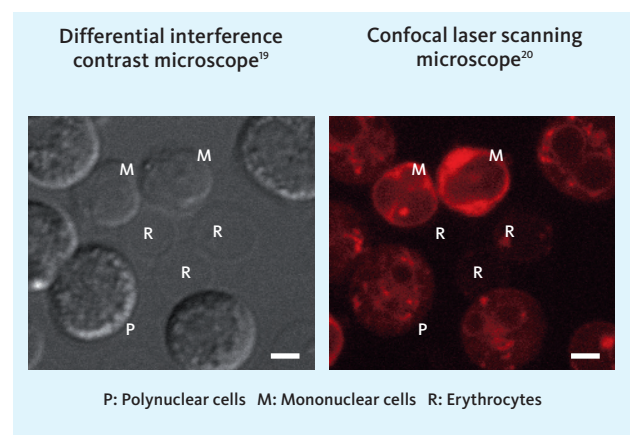
Scanning electron microscopic¹⁵ images



Bar = 1µm

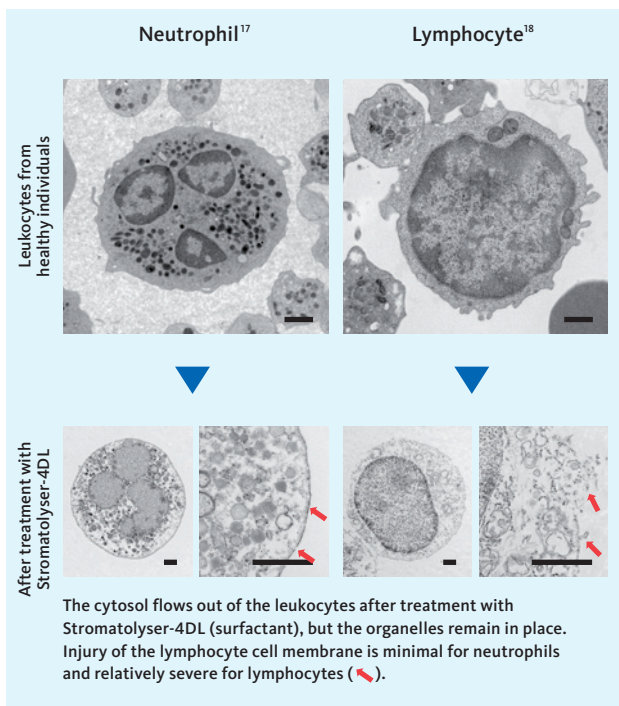
Fluorescence staining with Stromatolyser-4DS

When observed under a confocal laser scanning microscope, the fluorescence-positive area of the mononuclear cells (M) showed a ring-shaped form along the nucleic acid after treatment with Stromatolyser-4DL (surfactant) and Stromatolyser-4DS (fluorescent dye). At the same time, clear staining of nucleoli²¹ was noted. Polynuclear cells (P) showed weak diffuse staining across the entire cells, accompanied by staining of organelles. Erythrocytes (R) underwent rapid hemolysis after treatment with the reagent, showing no fluorescence detectable under a confocal laser scanning microscope.



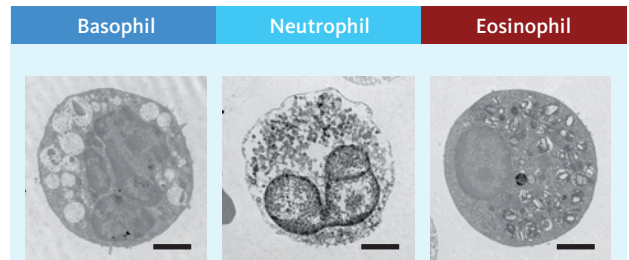
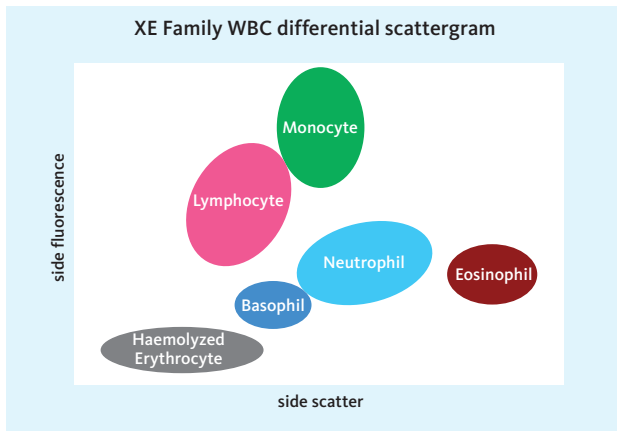
Bar = 2µm

Transmission electron microscopic¹⁶ images



Bar = 1µm

Leukocyte differentiation scattergrams obtained with XE Family and cell imaging

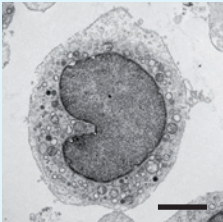
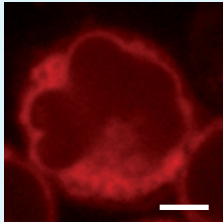


Granulocytes (a type of leukocyte) are primarily fractionated on the basis of the intensity of side scatter. Regarding eosinophils²⁴, it is known that the organic acids contained in Stromatolyser-4DL (surfactant) react with the eosinophil granules, resulting in firmly insoluble granulocytes. For this reason, eosinophils form a cluster with a high intensity of side scatter. Basophils²⁵ undergo degranulation under the influence from Stromatolyser-4DL, forming a cluster with a low intensity of side scatter. In case of neutrophils, the cytosol flows out of the cells following treatment with the same reagent, but organelles such as granules remain in place. On the scattergram, neutrophils form a cluster midway between the cluster of eosinophils and that of basophils.

Bar=2µm

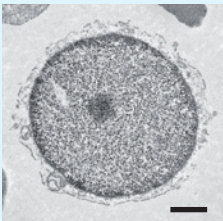
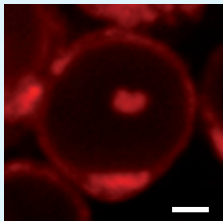
The morphological findings from the present study, using leukocyte bioimaging technology, endorsed the validity and accuracy of leukocyte differentiation measurement with XE Family.

Monocyte

Monocytes²² are rich in organelles. Many structures remain inside the Monocytes even after treatment with Stromatolyser-4DL (surfactant). For this reason, side scatter from monocytes seems to be more intense than that from lymphocytes. Because Stromatolyser-4DS (fluorescence dye) can stain many organelles remaining in the cells, clusters are formed in areas emitting intense fluorescence.

Lymphocyte

Stromatolyser-4DL (surfactant) is likely to induce permeabilization²³ across the cell membrane of lymphocytes, thus allowing Stromatolyser-4DS (fluorescent dye) to be rapidly introduced into the cells. For this reason, the intensity of fluorescence from lymphocytes seems to be slightly higher than that from granulocytes. However, since lymphocytes are poor in organelles, the intensity of side scatter and the intensity of fluorescence from lymphocytes form a cluster at levels lower than those for monocytes.

Terminology

1 Surfactant

A collective term for substances having both a hydrophilic portion (hydrophilic group) and a lipophilic portion (lipophilic and hydrophobic groups) within the molecule. Sysmex's reagent for leukocyte differentiation (Stromatolyser-4DL) contains cationic and nonionic surfactants, which act on the cell membrane and form ultramicroscopic pores in the membrane.

2 Fluorescent dye

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. Sysmex's reagent for leukocyte differentiation (Stromatolyser-4DS) contain fluorescent dyes of the polymethine family, which primarily stain the nucleic acids and organelles.

3 Flow cytometry

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. The Sysmex automated haematology analysers are based on this principle.

4 Bioimaging technology

A technology for observation of the distribution and kinetics of targets in cells or tissues marked with dyes, fluorescent dyes or colloid gold (used for electron microscopy).

5 Flow cell

A central unit of a flow cytometer in which cells are dispersed in a fluid flow through a small nozzle and are detected with laser, etc.

6 Stromatolyser-4DL

Sysmex's reagent for automated leukocyte differentiation, primarily made of cationic and nonionic surfactants. This reagent induces haemolysis of erythrocytes and formation of ultramicroscopic pores in the leukocyte cell membrane.

7 Stromatolyser-4DS

Sysmex's reagent for automated leukocyte differentiation, containing polymethine dyes which are excited by 635 nm laser. It primarily stains nucleic acids and organelles.

8 Nucleic acid

A macromolecule found in organisms. Can be divided into DNA and RNA. DNA is associated with genetic information in nuclei, while RNA is involved in expression of genetic information.

9 Organelle

Includes mitochondria, endoplasmic reticulum, Golgi apparatus, etc.

10 Side scatter

Laser (635 nm) scattered in the right-angle 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).

11 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 to the fluorescent dye.

12 Scattergram

Graphic representation of optical information of cells collected with a flow cytometer. Physical and chemical properties of cells are presented.

13 Leukocytes

Includes neutrophils, eosinophils, basophils, lymphocytes, and monocytes.

14 Cluster

A group of cells with similar physical and chemical properties formed on the scattergram.

15 Scanning electron microscope

A microscope allowing observation of the ultramicrostructure of the cell surface. A special metallic film is formed on the cell surface, and electron beams are applied to it for observation of the cell surface.

16 Transmission electron microscope

A microscope allowing observation of the ultramicrostructure inside cells. The cells are made into thin slices (70 nm) and electron beams are applied to them for visualization of electrons.

17 Neutrophil

A type of granulocytes, accounting for about 40–60% of all leukocytes. Contains granules which are positively stained with neutral dyes. Plays an important role in host defense through phagocytosis of bacteria, etc. and disinfection activity.

18 Lymphocyte

Accounts for about 25% of all leukocytes. Can be divided into NK cell, B cell (B lymphocyte), T cell (T lymphocyte), etc. Plays an important role in the immune system.

19 Differential interference contrast microscope

A microscope capable of three-dimensional observation of cells through interference of ray, making use of polarization.

20 Confocal laser scanning microscope

A microscope with laser serving as a light source, capable of achieving high spatial resolution not possible with a fluorescence microscope. Also capable of providing sectional images of cells stained with fluorescence.

21 Nucleolus

A region with high molecular density within the nucleus of eukaryote cells. A place for transcription of ribosomal RNA and construction of ribosomes.

22 Monocyte

Accounts for about 3–8% of all leukocytes. Plays an important role in initiation of anti-infection immune activity. Mobile through amoeba-like motion. Takes up bacteria and other foreign particles and digests them with intracellular enzymes.

23 Permeabilization

A manipulation to create a hole in the cell membrane which partitions the areas inside and outside the cell, to allow introduction of the target substance into the cell. In this case, Stromatolyser-4DL (surfactant) is used to create an ultra-microscopic pore in the cell membrane.

24 Eosinophil

Accounts for about 0–5% of all leukocytes. When specimens stained by ordinary methods are observed, the cells are filled with relatively large, round granules of similar features stained orange-red color with eosin. Can cause damages to bacteria and parasites.

25 Basophil

Accounts for about 0–2% of all leukocytes. Round cells with a size slightly smaller than neutrophils. Stained dark purple color with aniline blue.

Reference

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