DOUBLE HYDRODYNAMIC SEQUENTIAL SYSTEM

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The Double Hydrodynamic Sequential System (DHSS) technology is a HORIBA medical patent. Its significance is to ensure a linear flow of cells through electrical impedance method and optical measurement which helps in well differentiation of WBCs sub-population, along with Platelets. Double Hydrodynamic focusing is a technique used to provide accurate results from flow cytometer. DHSS helps

1) In measurement of cell size by impedance,
2) Analysis of complexity of cell (by diffraction and optical light extinction (fig1.1)
3) Combination of flow cytometry and cytochemistry technologies makes analysis more precise and provides accurate results.

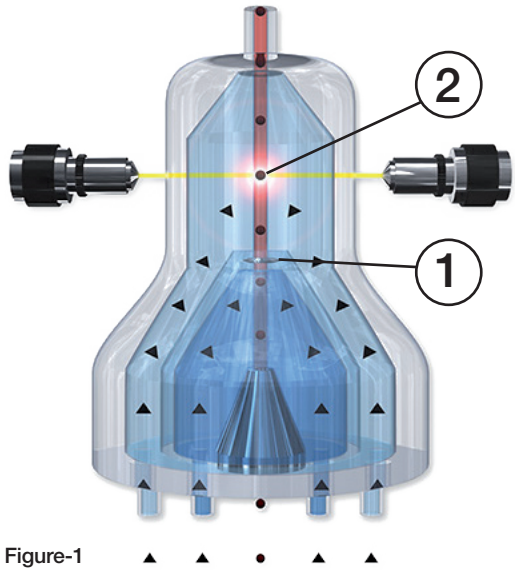
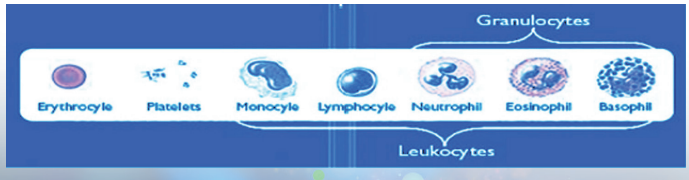


Figure-1



In approach to blood analysis, Specialized HORIBA Medical reagents prepare cells for better differentiation among themselves.
HORIBA Medical Analyzers uses a combination of optical measurement and electrical impedance for counting & analyzing the cells as they pass through a micro aperture electrode system. Electrode impulse is then used to count the number of cells, measure the size and distribution of leucocytes, erythrocytes and thrombocytes as shown in the figure above.

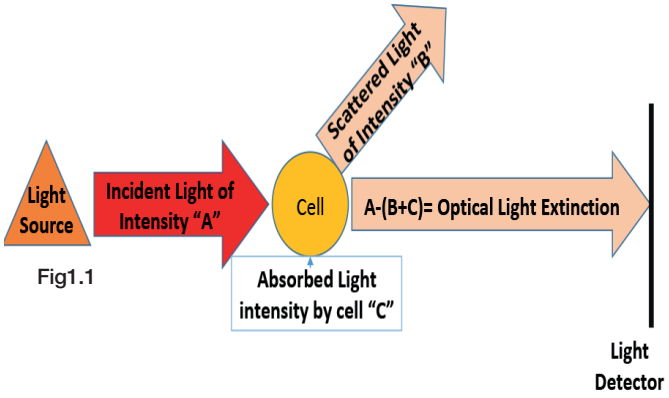


Fig1.1

LMNE Measurement Principles

HORIBA Medical special reagent Nucediff lyse RBC and stabilizes the leucocytes (WBC) in their native forms. Each cell is then measured both in optical extinction (internal structure) and resistivity (volume) which allows total nucleated cells count and differential count of lymphocytes, monocytes, neutrophils, eosinophils, atypical lymphocytes, immature populations and erythroblasts.

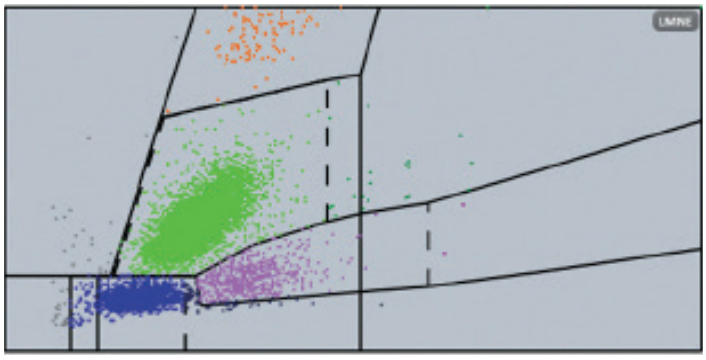
Keywords: Cell Complexity, Cytochemistry, Flow cytometery, Resistivity, Optical light extinction

The LMNE detection principle is based on the Double Hydrodynamic Sequential System «DHSS» flowcytometry.
Two characteristics for each cell passing through this system are determined: volume and Optical light extinction
(HORIBA Medical patent).

1. The cells go through the aperture one by one (1 in Fig.1) to be counted and measured by electrical current (impedance changes). This is the resistive measurement for cell volume.
2. Then each one crosses the light beam that arrives at a 0° angle (2 in Fig.1) determining the cells complexity.
- Some part of the light is absorbed by the cell content,
- Major part is deviated by the cell structure (scattered light),
- Rest of the part passes through or around the cell.

Matrix and Cells Description
From the extinction and resistive measurements of the leukocytes, a matrix is developed with cell volumes on the X-axis and extinction on the Y-axis. Study of the matrix image allows a clear differentiation of leukocyte sub populations.

The cell population thresholds (some of them mobiles and the others fixed) give the normal limits for the normal leukocyte morphologies. Changes in the morphology of a specific population is indicated on the matrix by a shift in the corresponding population.
The fixed thresholds (Fig.2) appear in black and the mobile thresholds appear in red in the picture below. The blue thresholds follow the red ones when adjusting the matrix.
The matrix enable the calculation of the leukocytes population percentage following this figure:



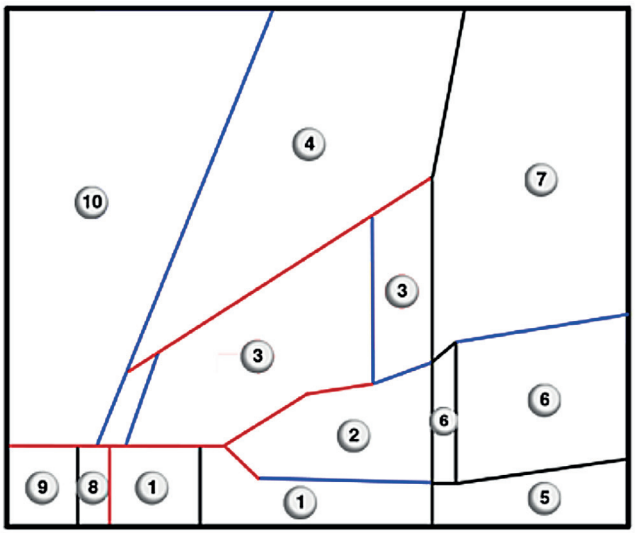


Figure-2

1=Lymphocytes;
2=Monocytes;
3=Neutrophils;
4=Eosinophils;
5=IML(Immature Lymphocytic cells);
6=IMM (Immature Monocytic cells);
7=IMG(Immature Granulocytic cells);
8=Erythroblasts or Platelet aggregates;
9=Background Noise Low;
10=Background Noise High

Keywords: Matrix, Leucocyte sub population morphologies

Erythroblasts (NRBC)
Erythroblasts are immature red blood cells with a nucleus.
The flow-cytometer detects the nuclei of the NRBC cells liberated after the Nucediff lyses their membranes. Because of their small size and low absorbency, they are placed on the left part of the lymphocyte population on the matrix (box #8). In this zone, we can also find platelet aggregates. The instrument can differentiate them from the erythroblasts thanks to specific algorithms.
In all cases, NRBC and platelet aggregates are not included in WBC counts.

Cellular Morphology with DHSS:
In the HORIBA Medical analysers, the extinction measurement is made using low coherence optical source.
The low coherence optical source is characterized by the different spectral and spatial components of the light beam.
This measurement reveals the morphology of the different cells organelles on the one hand, and intra-cytoplasmic characteristics on the other hand.
The high numeric aperture of the light beam allows the observation of cells.

This measurement is less sensitive to cell anisotropy, and its position or orientation in the beam (Fig.3), and gives information depending on the intrinsic characteristics of the cell.

Low coherent Light Source:
In polychromatic light using a beam of low spatial coherence (divergent beam), the measurement is less sensitive to the position or orientation of the cell in the beam.

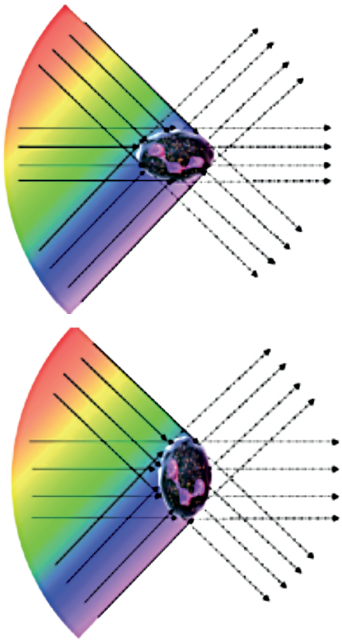
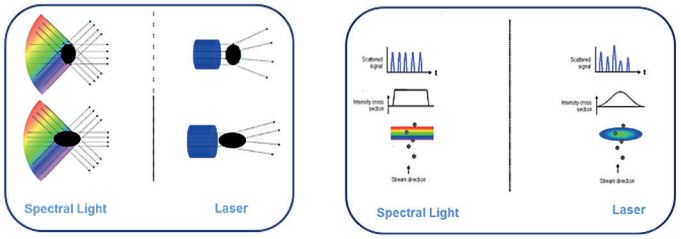


Fig 3: the measurement is slightly affected by the orientation or by the cell position.

Why Low coherent Light and not a Laser Light?
Giving the complexity of the structure and morphology of the leukocytes, the identification and counting method combining cytochemistry and low coherent light measurement (low coherence illumination) proves to be more discriminating and more robust compared to laser methods.



A low coherent light allows a cell analysis under multiple angles. Light beam is perfectly homogeneous at the measurement point (Köhler).
The measurement is not sensitive at the cell position within flux and so more reproducible, repeatable and accurate.

	Laser	Low Coherent Light Lamp
Advantage	High power in flow cell (90% of light source) Suitable for fluorescence	Flatness of beam intensity profile . Very Low noise (high frequency)
Limitation	Noisy light , speckle, Difficult to use for precise volume measurement. Gaussian light shape	Low efficiency (0,0004 %) Electric consumption, thermal emission. Not suitable for Fluoresence.

Keywords: Low Coherent Light, PLT Ox

The optical measurement done on Yumizen analyzers allow to provide more accurate information on the volume, nucleo-cytoplasmic ratio and internal structure of each cell, and this robustness is particularly useful for abnormalities identifications.

Optical Platelet Extinction Analysis
Similar to WBC analysis, Platelets are counted with utilization of Double hydrodynamic sequential system when samples are processed on PLT Ox mode. PLT Ox mode differentiate Platelet from RBC population on the basis of refractive index.

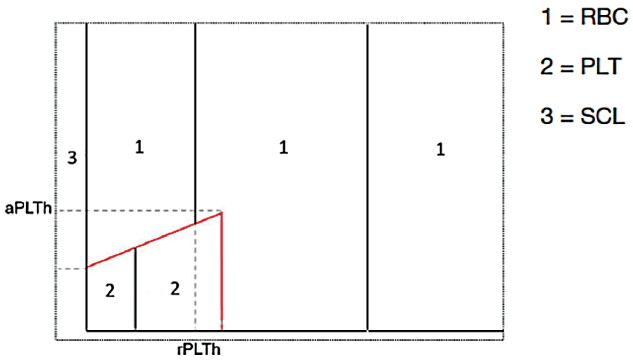
The measurement principle is similar to the LMNE one.

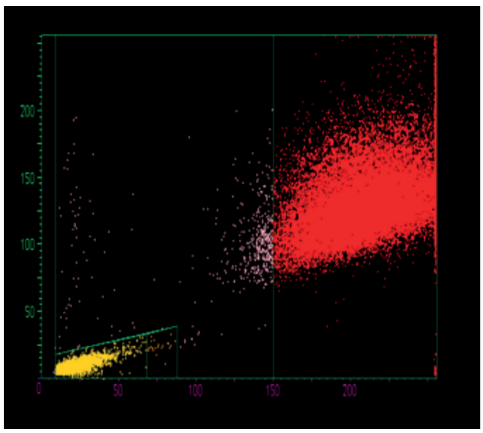
1. After the dilution, the solution is transferred from the chamber to the optical bench using an aspiration, before being analyzed in the measuring chamber. The dilution is sleeved with a double hydrodynamic sleeving and passes through the aperture of the LMNE flowcell and the optical window. Each cell is measured both in resistivity (volume) and absorbency (cytochemistry).
2. From these measurements, a matrix is drawn up with volumes on the X-Axis and optical extinction on the Y-Axis.

PLT Ox:
This parameter corresponds to the percentage of platelets relative to the RBC identified on the matrix, multiplied by the count of RBC identified in the chamber.
No need to calibrate this parameter since it relies on the calibration of the RBC.
At the same time, the instrument runs a RBC/PLT Low Value cycle.

How does Double Hydrodynamic Sequential System improve the accuracy of Hematology Analysis?
Hydrodynamic focusing uses sheath flow to control the sample rate inside the aperture and direct the sample flow along the central axis of the aperture. Here in HORIBA Double Hydrodynamic Sequential System is used to prevent edge effects from altering the results and helps to align particles to the same orientation.

Matrix





What is Edge effect?
The presence of stronger electric gradients near the edges of an electric field than at the center can influence accuracy, it can distort the size of the electrical pulse, resulting in an overestimation of a particle's size. This occurs when particle pass through the micro-aperture at different positions.
It is further complicated by the particle's orientation through the aperture.

The use of DHSS avoids many of the potential problems inherent in a aperture system. The sample stream is surrounded by a sheath fluid, as it passes through the central axis of the aperture. Laminar flow allows the central sample stream to narrow sufficiently to separate and align the cells into single file for passage through the sensing zone.
The outer sheath fluid eliminates recirculation of cells back into the sensing zone.

Keywords: Edge Effect, Cell recirculation

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