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Automated blood cell count

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Disclaimer: *As automated blood cell counts are nowadays exclusively performed on commercial platforms, multiple images used in this presentation are from commercial origin. These do not reflect any preference or quality judgement and are mainly intended to illustrate general principles.*

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- **Introduction**
- **Technical** details and **principles** of automated hematology Analyzers
    - Hb
    - PLT/RBC
    - WBC and differentiation
- **Automated** systems vs **microscopy**
- **Workflow organization** in a clinical laboratory

Introduction

What are we talking about?

Reimbursed parameters

- Hemoglobin
- Thrombocytes
- Hct/RBC
- WBC
- Differentiation
- Reticulocytes

(Hemoglobin, Thrombocytes, Hct/RBC, WBC: "CBC")

'Associated' parameters

- MCV, MCH, MCHC
- MPV
- Immature reticulocyte fraction
- Immature platelet fraction
- …

Calculated parameters

$$\mathrm{MCV\,(fl)} = \frac{\mathrm{PCV\,(l/l)} \times 1000}{\mathrm{RBC\,(cells/l)} \times 10^{-12}}$$

$$\mathrm{MCH\,(pg)} = \frac{\mathrm{Hb\,(g/l)}}{\mathrm{RBC\,(cells/l)} \times 10^{-12}} \text{ OR } \frac{\mathrm{Hb\,(g/dl)} \times 10}{\mathrm{RBC\,(cells/l)} \times 10^{-12}}$$

$$\mathrm{MCHC\,(g/dl)} = \frac{\mathrm{Hb\,(g/l)}}{\mathrm{PCV\,(l/l)} \times 10} \text{ OR } \frac{\mathrm{Hb\,(g/dl)}}{\mathrm{PCV\,(l/l)}}$$

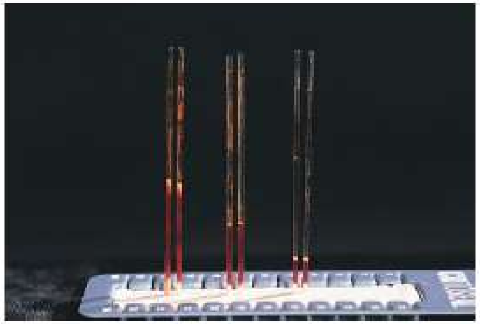
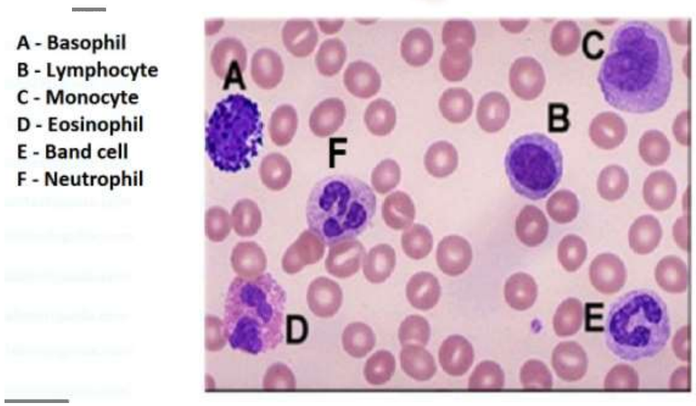
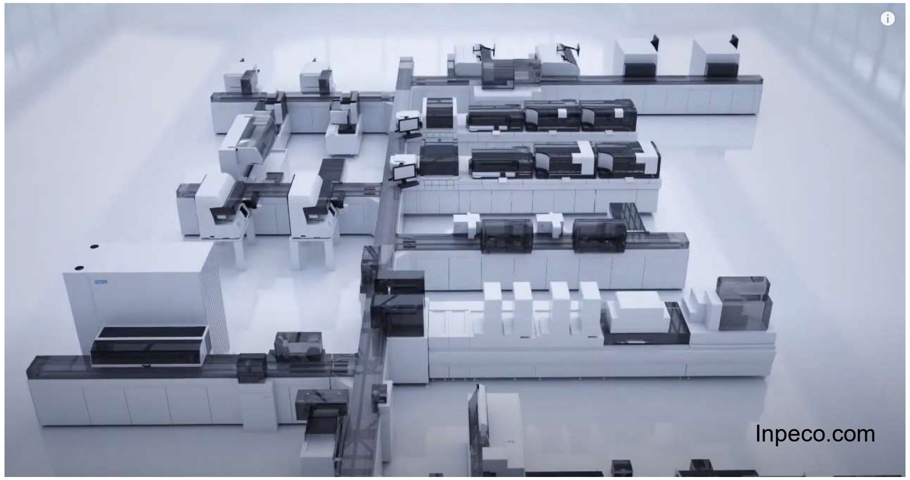


Fig. 2.2 Measurements of packed cell volume (PCV) by the microhaematocrit technique; paired tests from three patients are shown.



Evolution

Continuous evolution



> Ann Lab Med. 2020 Mar;40(2):122-130. doi: 10.3343/alm.2020.40.2.122.

Performance Evaluation of Body Fluid Cellular Analysis Using the Beckman Coulter UniCel DxH 800, Sysmex XN-350, and UF-5000 Automated Cellular Analyzers

> Int J Lab Hematol. 2008 Dec;30(6):536-42. doi: 10.1111/j.1751-553X.2007.00996.x.

Performance evaluation and relevance of the CellaVision DM96 system in routine analysis and in patients with malignant hematological diseases

Clinical Trial > Int J Lab Hematol. 2020 Dec;42(6):744-749. doi: 10.1111/ijlh.13281. Epub 2020 Jul 8.

A new approach for diagnosing hematological malignancies using monocytosis workflow optimization and abnormal lymphocyte/blast flag of Sysmex XN series of blood count analyzers

ORIGINAL ARTICLE

WILEY ISLH International Journal of Laboratory Hematology

Performance evaluation of the automated nucleated red blood cell count of five commercial hematological analyzers

Observational Study > Medicine (Baltimore). 2020 Feb;99(7):e19096. doi: 10.1097/MD.0000000000019096.

Immature platelet fraction: A useful marker for identifying the cause of thrombocytopenia and predicting platelet recovery

> Clin Biochem. 2016 Nov;49(16-17):1292-1294. doi: 10.1016/j.clinbiochem.2016.08.020. Epub 2016 Sep 2.

Diagnostic efficiency of the Sysmex XN WPC channel for the reduction of blood smears

Advantages of automation and technical evolutions

- Major reduction in TAT
- Major decrease in CV% => more reliable results
- Sample throughput
- Smaller blood volumes
- Additional information (‘associated’ parameters)
- Pre-analytical control

	30/10	29/10
Hgb (g/dL)	8	12
Hct	32	42
RBC	3.5×10^6	5×10^6
WBC	6.5×10^3	8×10^3
TROC	220×10^3	250×10^3

- …

Manual processing


Full automation

Part 1: Technical details and principles of automated hematology Analyzers

General principles

- Each analyzer uses a **combination of detection principles** to separate and count the individual cells in blood, based on the unique properties of these cells (size, granularity, RNA-content,…)

- These detection principles are chosen to be **cheap, quick, reproducible, robust and automatable**

- Most of these properties are **not absolute specific for a cell-type** (eg CD41 based measurement of PLT vs size-based measurement)

- If cells shows **'abnormal' properties** (eg, giant thromobcytes, cells with increased metabolic activity,…), these may (or may not) behave differently in a specific measurement technique and **lead to spurious counts**.

- Designed to **count 'normal' cells** and **detect** presence of **abnormal cells** (morphology/FCM: identify and type abnormal cells)

Hemoglobin

- Colorimetry

Colorimetry

Reference-method: cyaanmethemoglobine method

- Stable cyano-Hb complex after RBC-lysis, measurement of absorption at specific wavelength
- Difficult to automate (=slow reaction)
- Need for toxic CN-chemicals

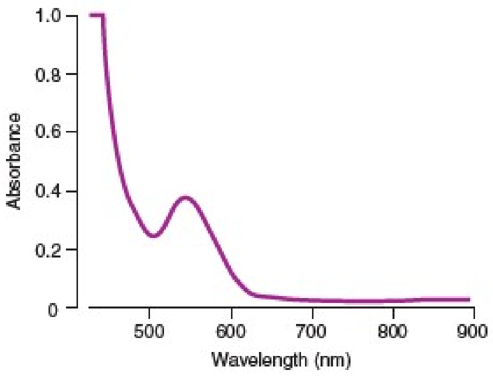


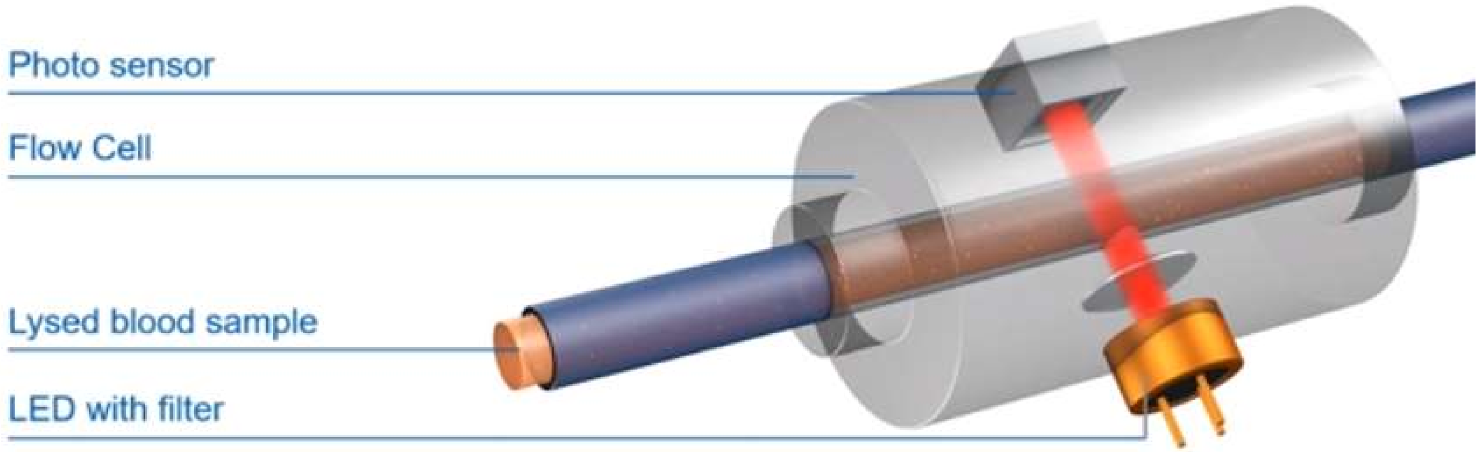
Fig. 2.1 Absorbance spectrum of cyanmethaemoglobin.

Blood cells, Bain

In routine practice: CN-free methods and reagents

Haemoglobin Measurement

- The haemoglobin concentration is determined from the absorbance measured
  by a photometric method at 555 nm.



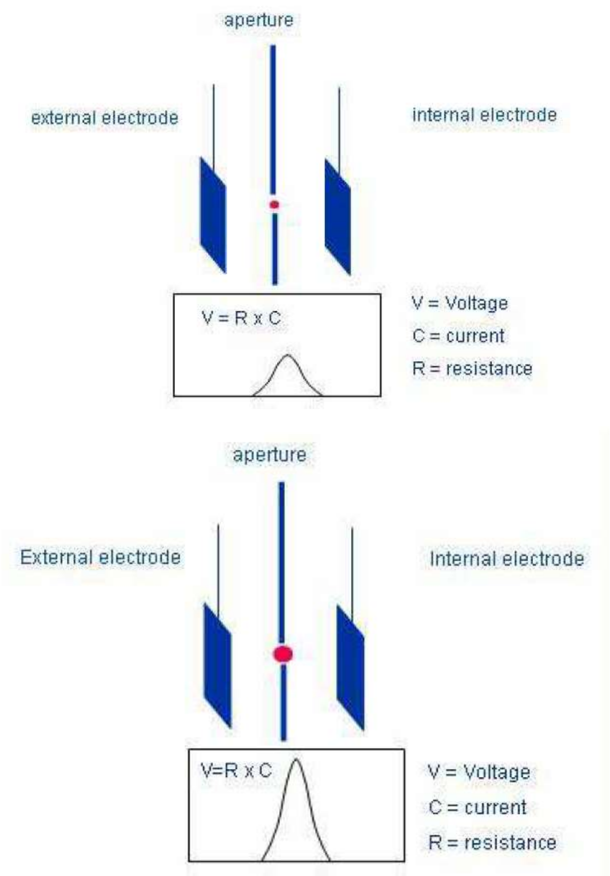
Interferentie by turbidity, eg lipemia

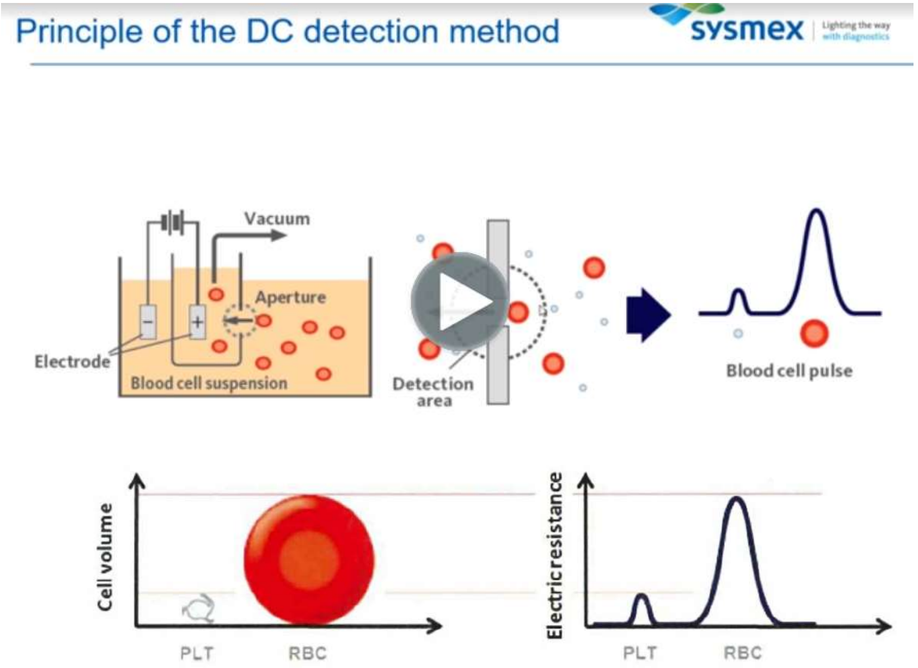
Bron: Sysmex

RBC-PLT

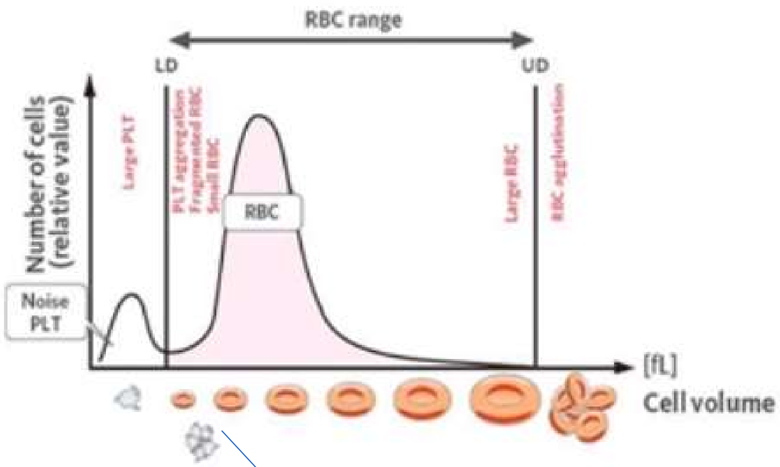
- Impedance
- Light Scatter
- Fluorescence

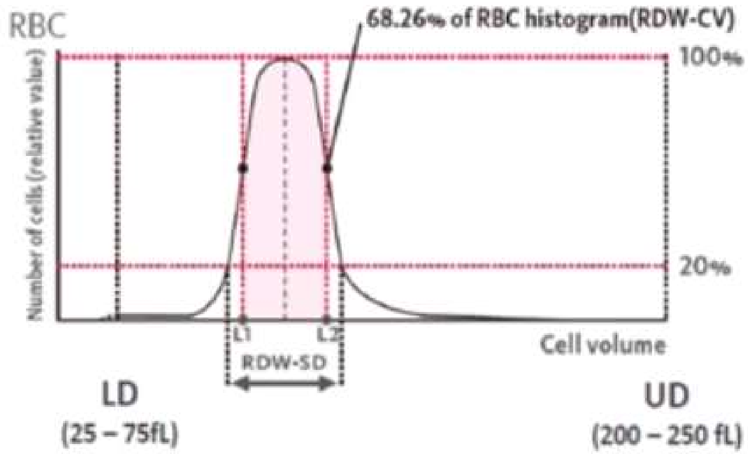
Impedance (RBC-PLT) (Sysmex, Abbott, Beckman)





RBC Histogram





RDW-CV: RBC distribution width coefficent of variation

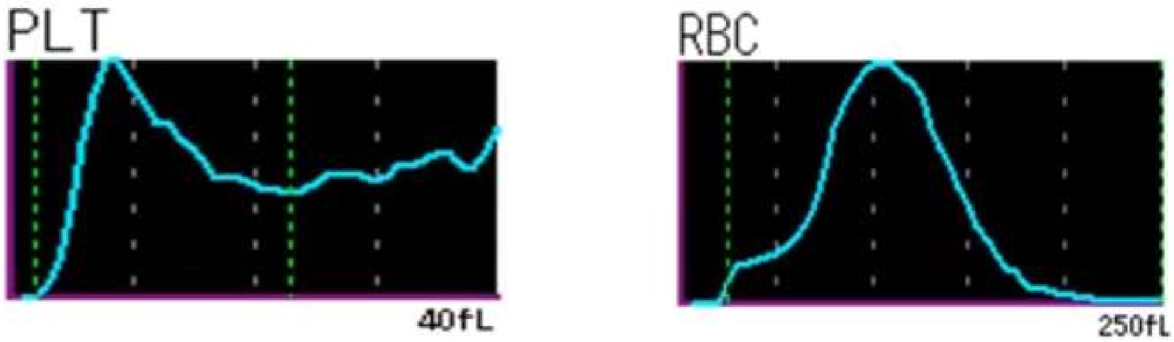
RDW-SD: RBC distribution width standard deviation

Measurement of particles with the size of RBC ≠ RBC

Bron: Sysmex

Impedance = ‘particle’ counter

Prone to interferences

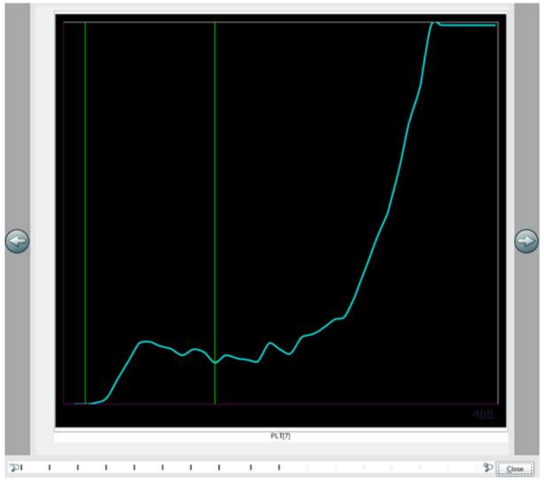


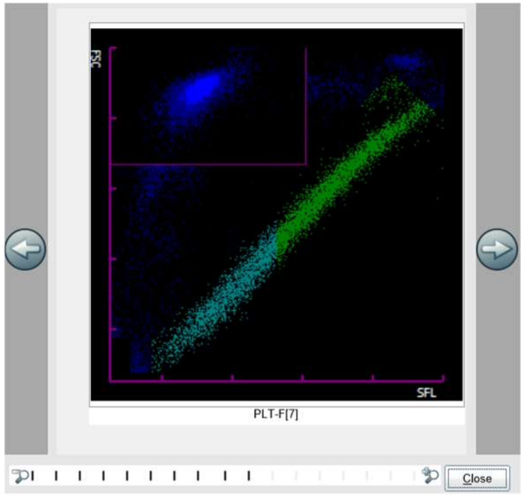
- Fragmentocytes
- Microcytes
- Giant trombocytes
- PLT aggregates.......

Cave: RBC >> PLT

Spurious PLT-count, Example 1

- PLT Abn distribution

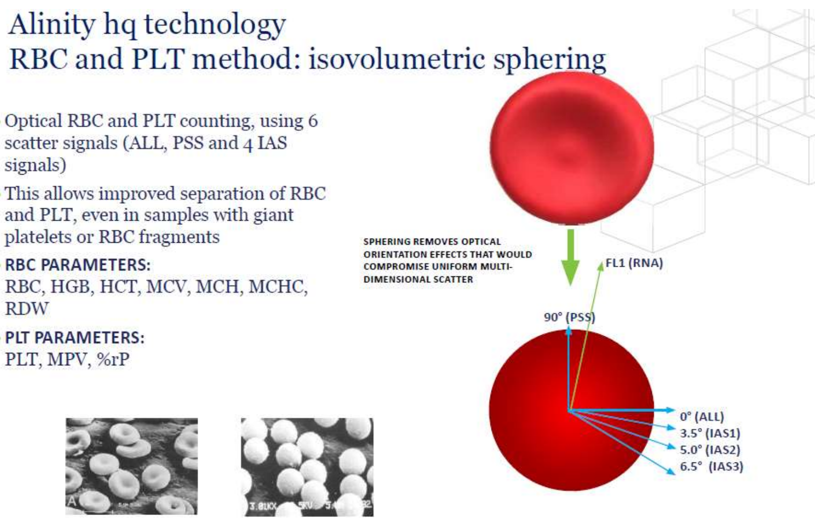


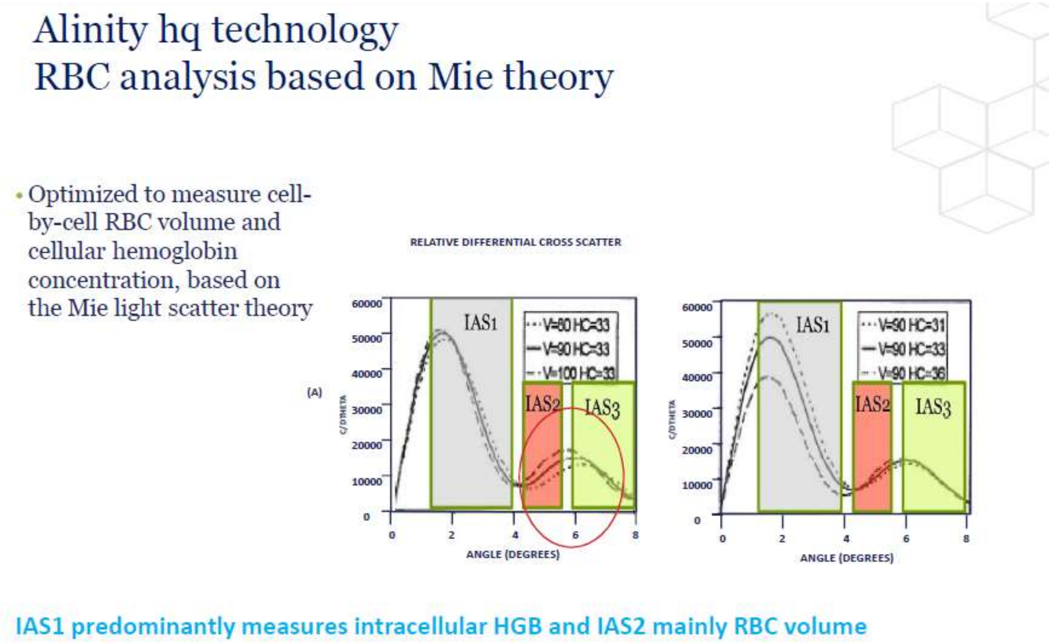


			PLT-F Research			
	A	7	PLT-F	76		10*3/µL
	A	7	H-IPF	50.1		%
	A	7	IPF#	47.3		10*3/µL
	A	7	PLT-F2	77		10*3/µL
	A	7	WBC-N	6.11		10*3/µL
	A	7	TNC	6.11		10*3/µL
	A	7	TNC-N	6.11		10*3/µL
	A	7	BA-N%	0.0		%
	A	7	BA-N#	0.00		10*3/µL
	A	7	MicroR	26.9		%
	A	7	MacroR	3.2		%
	A	7	PLT-I	26		10*3/µL
	A	7	PDW_RESEARCH	---- not measurable		fL
	A	7	P-LCR_RESEARCH	---- not measurable		%
	A	7	PCT_RESEARCH	---- not measurable		%

Underestimation of PLT-count by impedance method due to macrothrombocytes

Light scatter (RBC-PLT) (Siemens, Abbott)

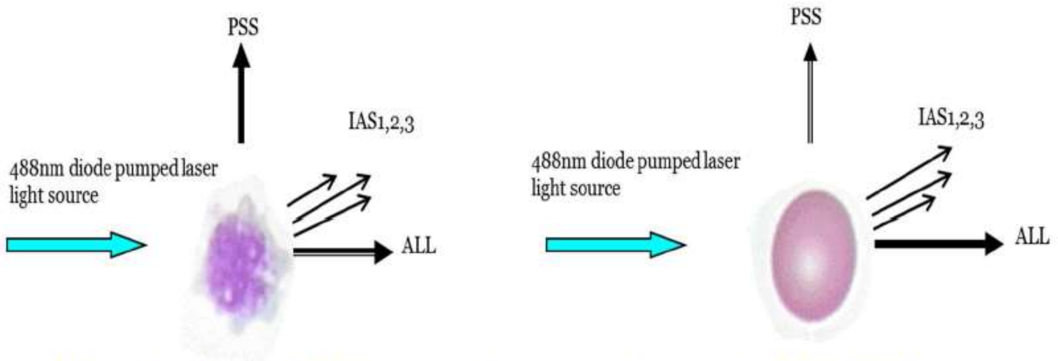




Bron: Abbott

Light scattering allows for better discrimination between PLT and RBC (fragments)

Alinity hq technology
PLT method: multidimensional light scatter

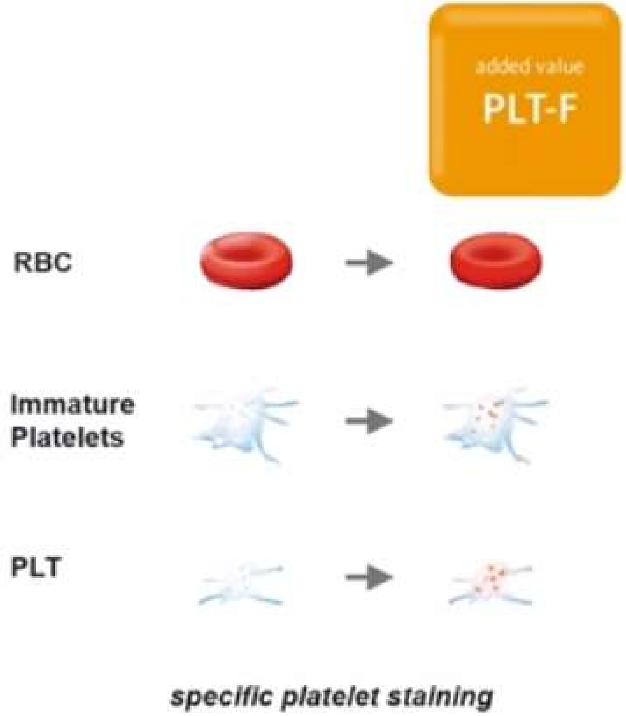


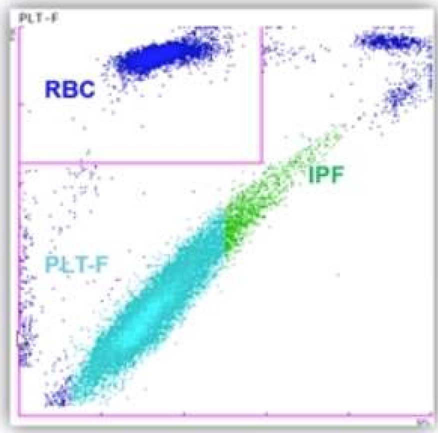
- When platelets and RBCs are similar in size (microcytic RBC, RBC fragments, large or giant platelets) electrical impedance or dual angle light scatter may demonstrate signal overlap
- With the implementation of multi-dimensional analysis, platelets and RBCs of similar size demonstrate unique signal signatures with the array of different angles of light scatter

Bron Abbott

Fluorescence (PLT) (Sysmex)

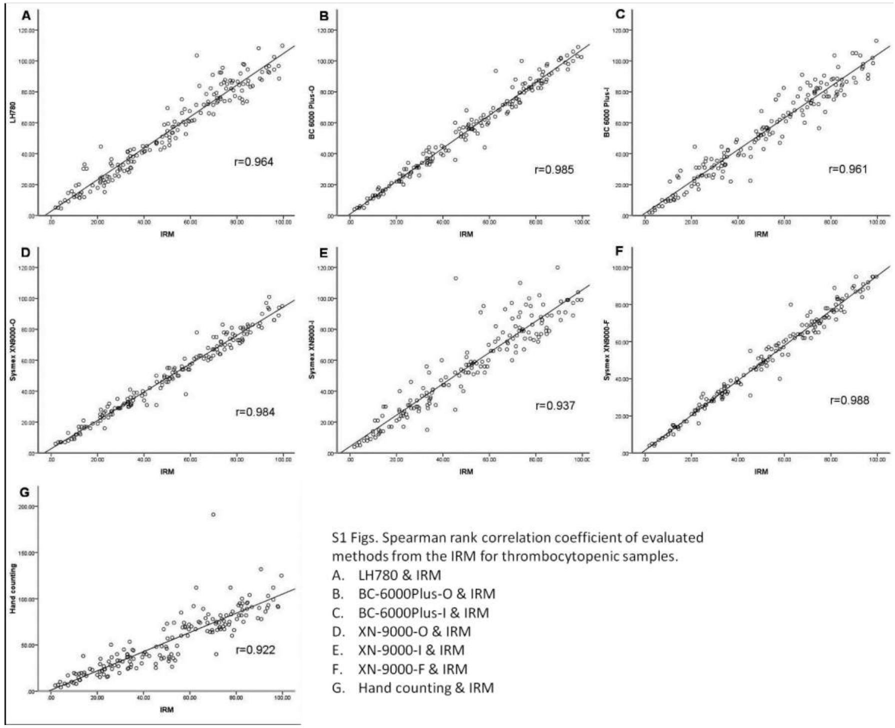
- Fluorocell PLT-F stains RNA in PLT by reagent component Oxazine
- Differentiation of populations by fluorescence intensity and size
- Reticulocytes and RBC are not stained



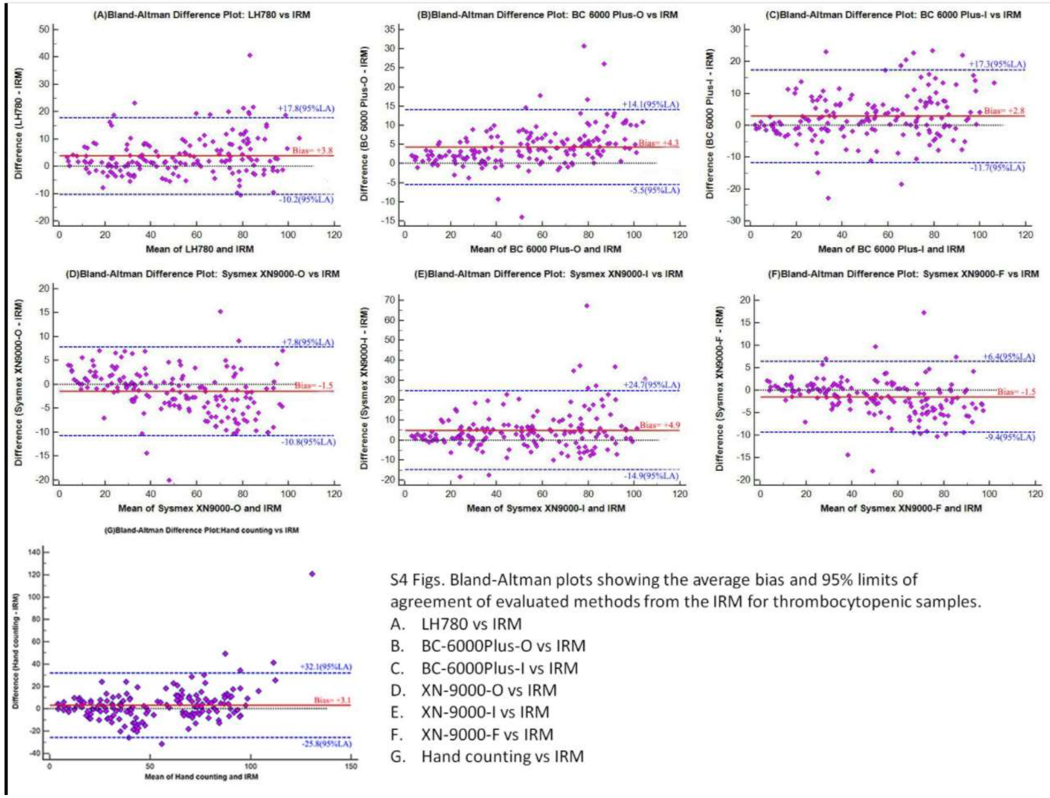


FSC: Forward Scattered Light
SFL: Side Fluorescence

Scattering, Impedance, Fluorescence: does it matter?



S1 Figs. Spearman rank correlation coefficient of evaluated methods from the IRM for thrombocytopenic samples.
A. LH780 & IRM
B. BC-6000Plus-O & IRM
C. BC-6000Plus-I & IRM
D. XN-9000-O & IRM
E. XN-9000-I & IRM
F. XN-9000-F & IRM
G. Hand counting & IRM



S4 Figs. Bland-Altman plots showing the average bias and 95% limits of agreement of evaluated methods from the IRM for thrombocytopenic samples.
A. LH780 vs IRM
B. BC-6000Plus-O vs IRM
C. BC-6000Plus-I vs IRM
D. XN-9000-O vs IRM
E. XN-9000-I vs IRM
F. XN-9000-F vs IRM
G. Hand counting vs IRM

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Compare the accuracy and precision of Coulter LH780, Mindray BC-6000 Plus, and Sysmex XN-9000 with the international reference flow cytometric method in platelet counting

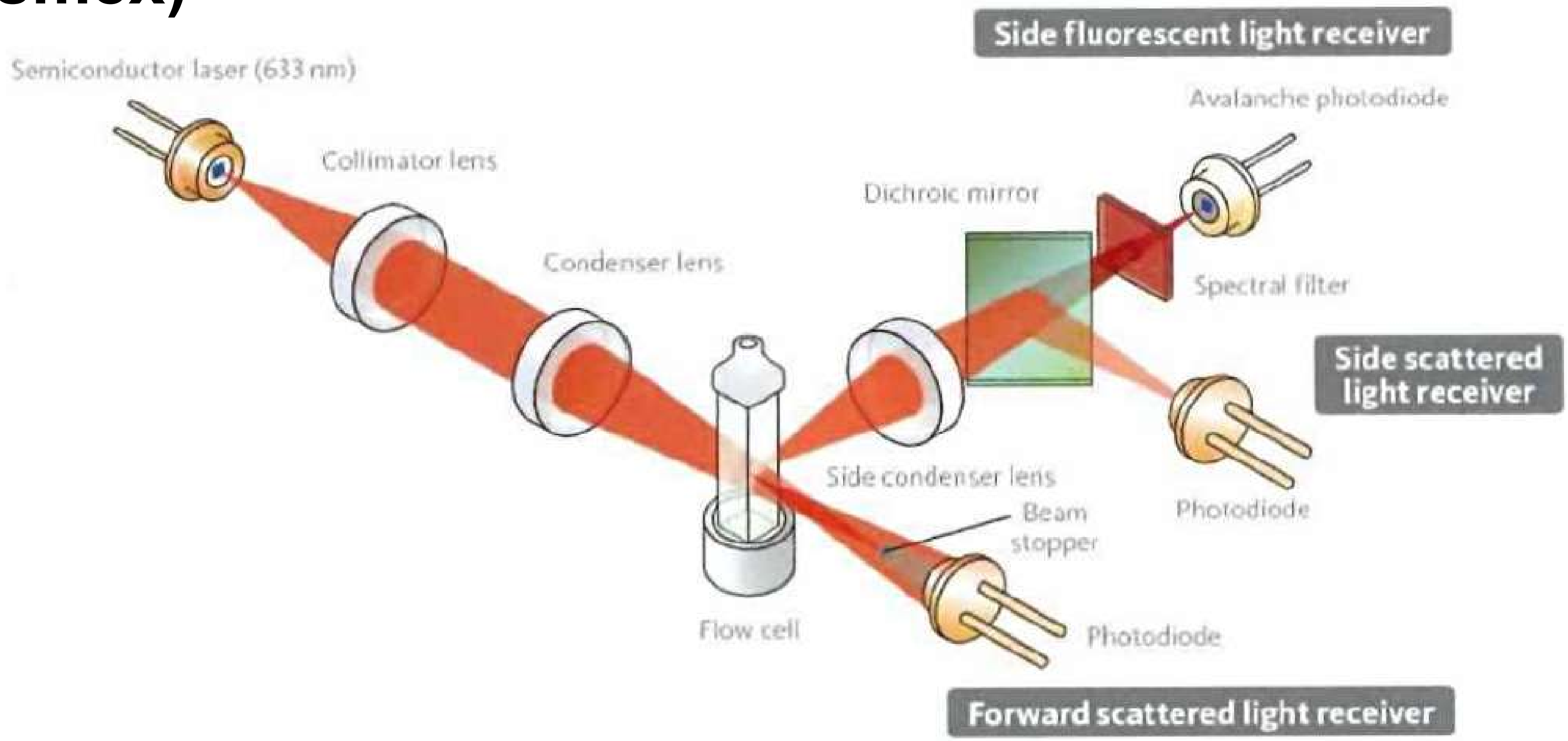
Yi Sun, Zuojian Hu, Zhili Huang, Huaping Chen, Shanzi Qin, Zhong Jianing, Siyuan Chen, Xue Qin, Yi Ye, Chengbin Wang

Published: May 24, 2019 • https://doi.org/10.1371/journal.pone.0217298

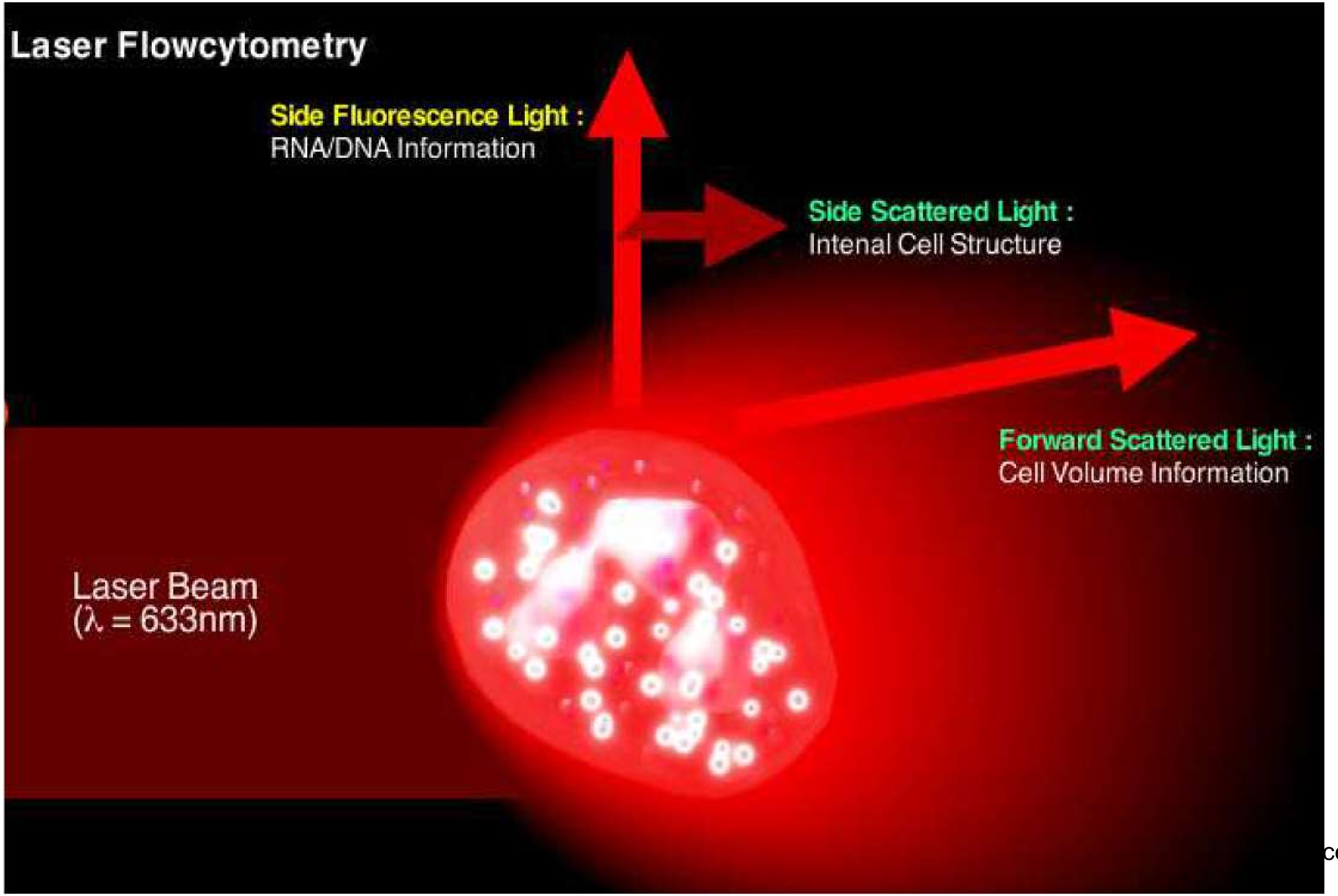
WBC

- Flow cytometry
- Light Scatter
- Impedance

Fluorescence flow-cytometry (WBC) (Sysmex)



Source: Sysmex



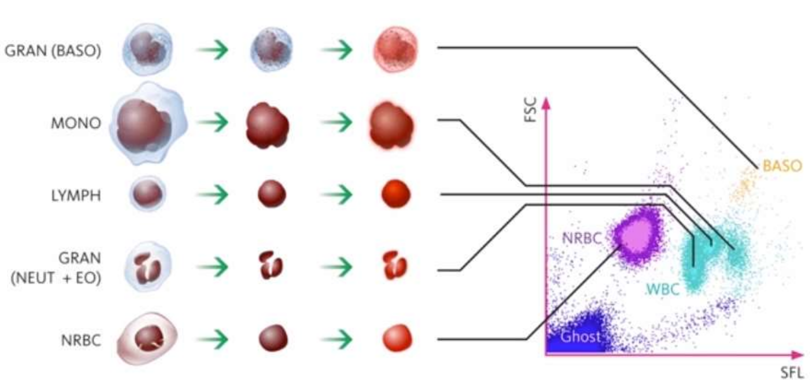
Laser Flowcytometry
Side Fluorescence Light :
RNA/DNA Information
Side Scattered Light :
Intenal Cell Structure
Forward Scattered Light :
Cell Volume Information
Laser Beam
(λ = 633nm)

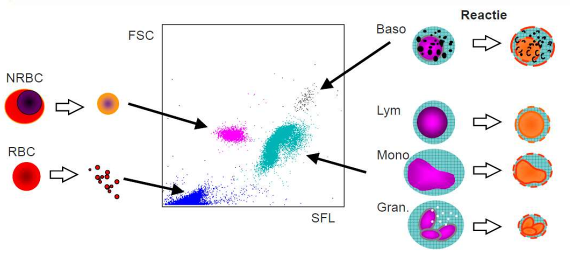
ce: Sysmex

Combination of:

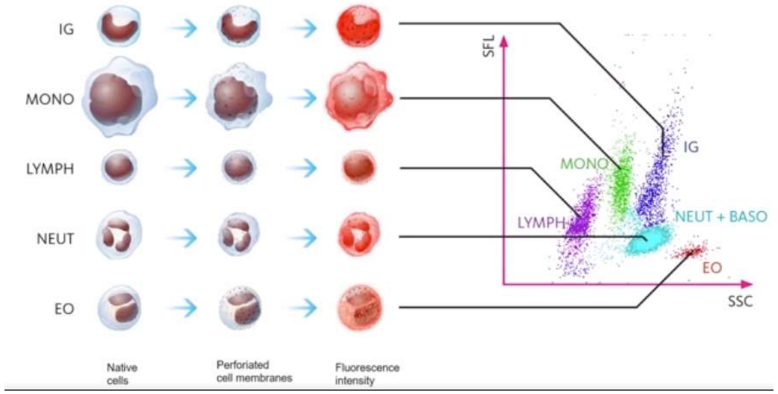
- -selective lysis
- -fluorescence intensity (dyes with RNA/DNA specificity)
- -FSC (size) en SSC (granularity)

Lysis and staining

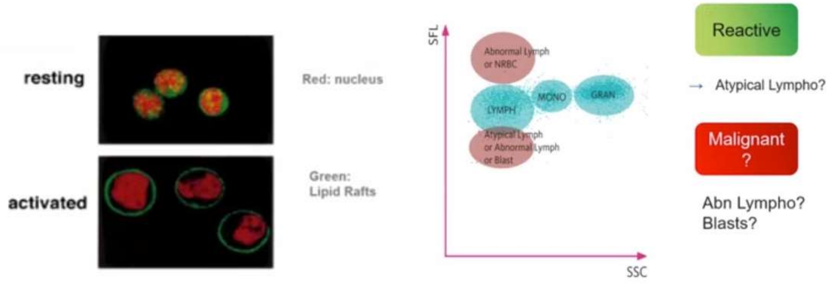




Perforation of cell membrane and staining



Perforation of cell membrane based on lipid content and staining



Source: Sysmex

Flagging WDF

sysmex Lighting the way with diagnostics

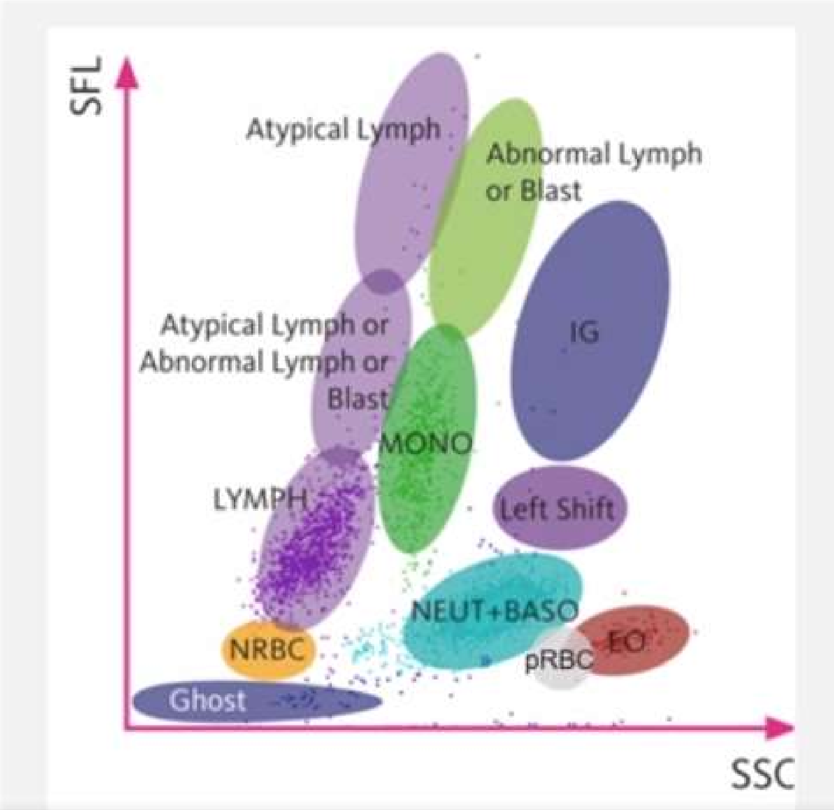
Abnormal messages:

1. WBC Abnomal scattergram
2. IG present*

Suspect messages:

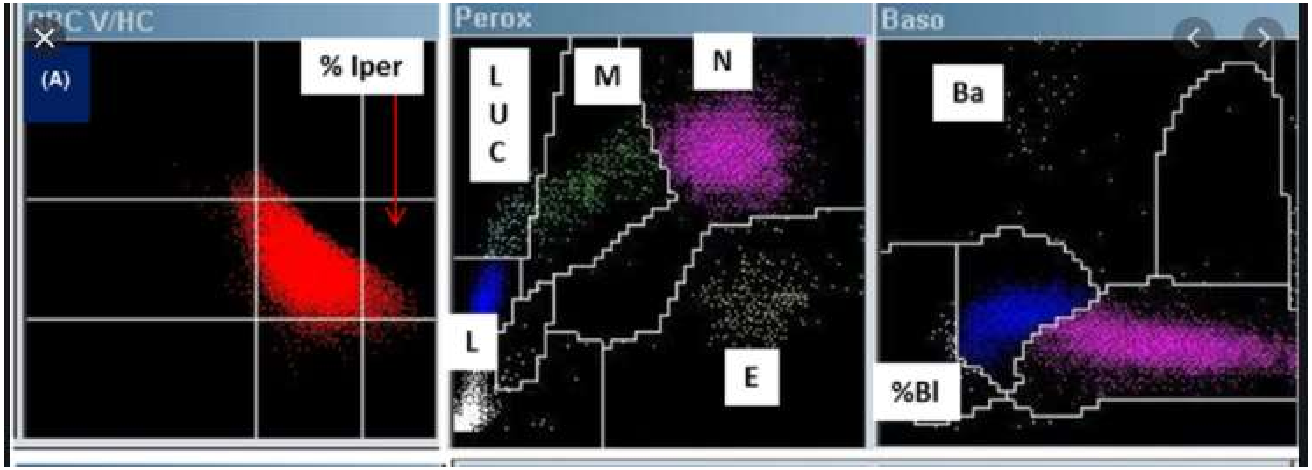
3. Left shift?
4. Atypical Lymph?
5. Blast/Abnormal Lymph?
6. iRBC?
7. PLT clumps?

* customizable by user



Source: Sysmex

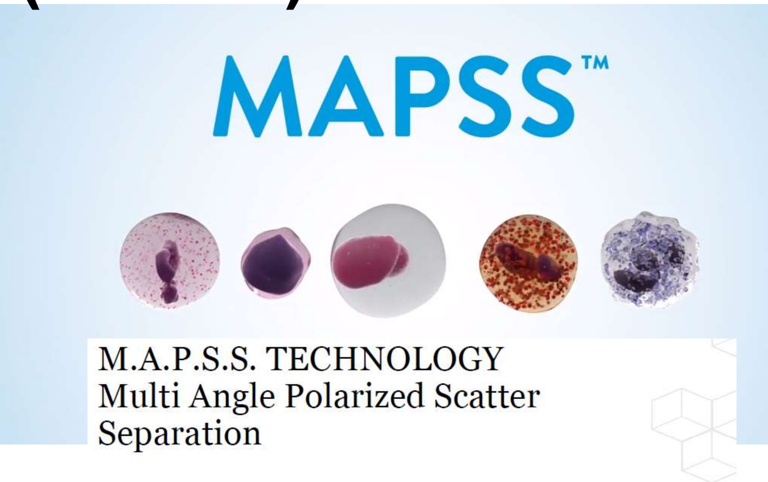
Cytochemistry - flow-cytometry (WBC) (Siemens)



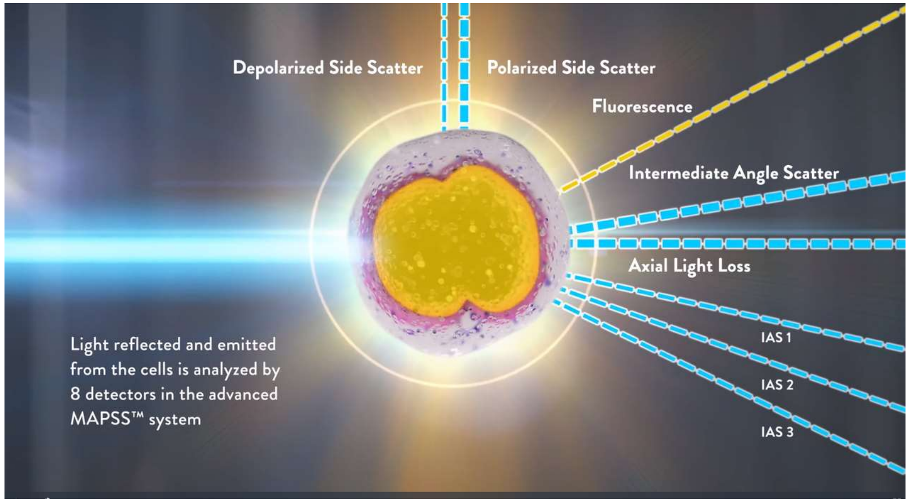
FSC vs
peroxidase

FSC vs SSC na selectieve
lyse

Fluorescence – Light Scattering (WBC) (Abbott)



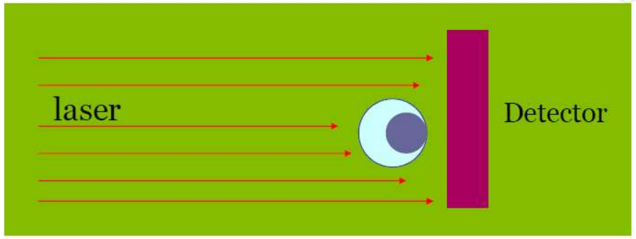
Counting and differentiating of blood cells in a near native state by use of their light scattering characteristics



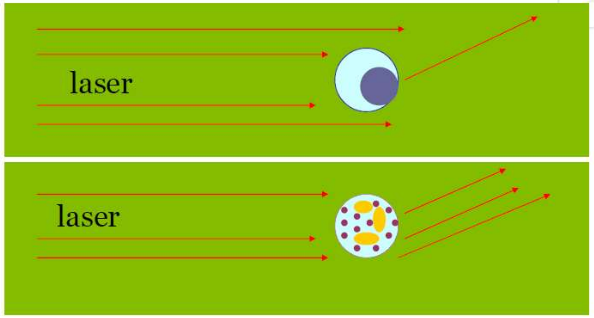
- The WBC reagent contains lytic agents and a proprietary membrane-permeable, fluorescent nuclear dye
- The fluorescent dye stains all nucleated cells (nucleic acid in WBC and NRBC) and does not stain RBC

Source: Abbott

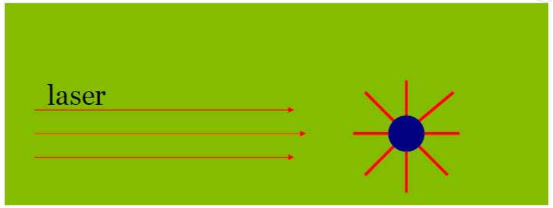
ALL = 0° = size



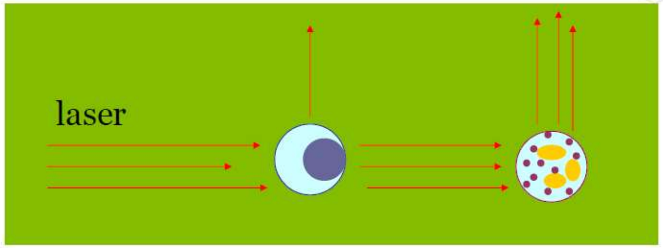
IAS = 7° = complexity/granularity



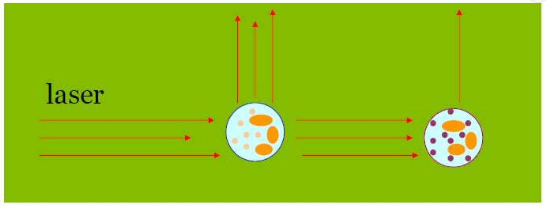
FL1 fluorescence = DNA



PSS = 90° polarization = lobularity

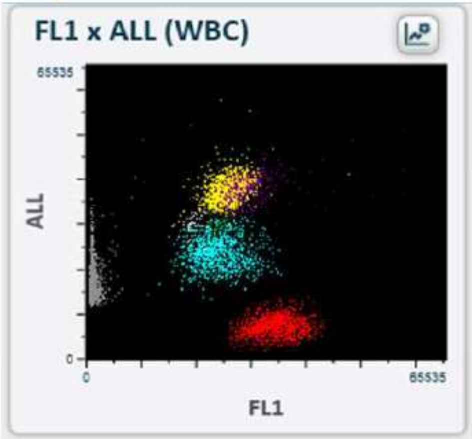


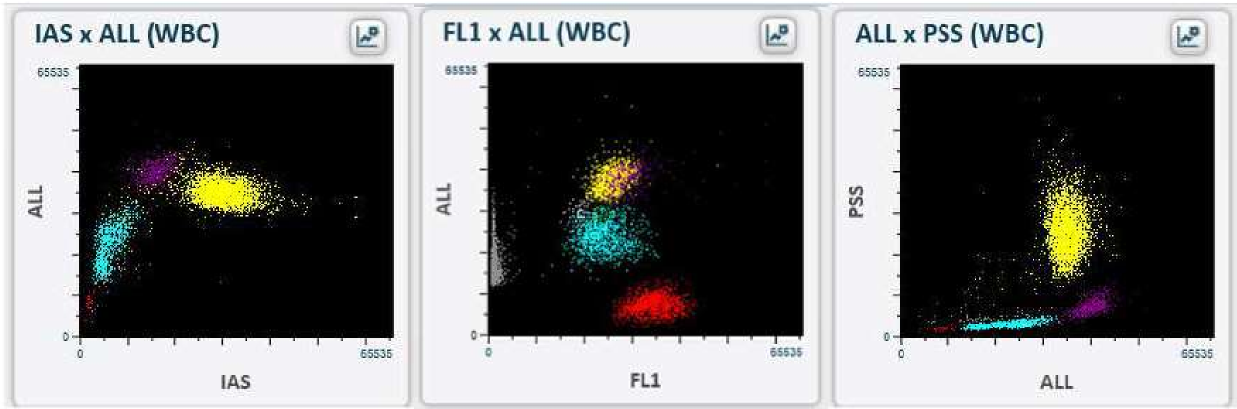
DSS = 90°depolarization = eosinophilic granularity



Source: Abbott

WBC method: nuclear fluorescent dye





FSC vs IAS

FSC vs fluo

SSc vs FSC

Neutrophilic granulocytes

Monocytes

Eosinophilic granulocytes

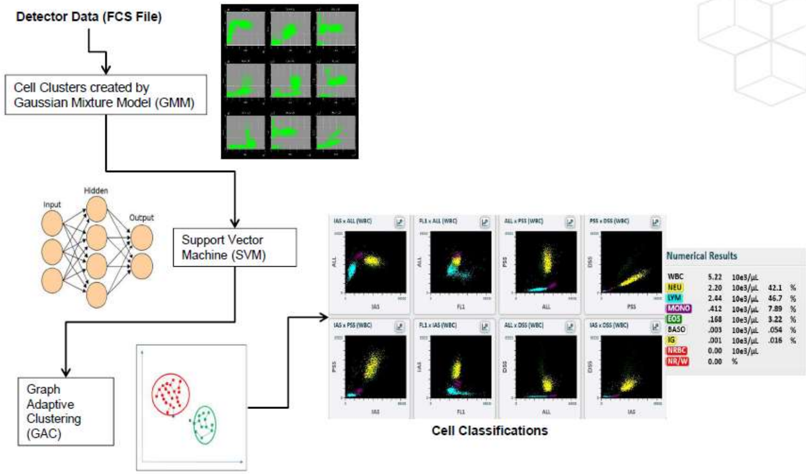
Basophilic granulocytes

Lymphocytes

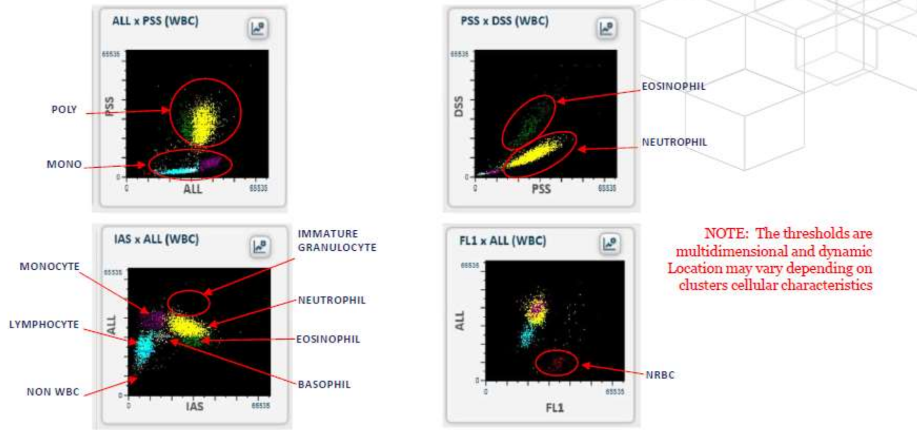
Nucleated Red Cells

Source: Abbott

WBC cluster analysis and classification



Primary WBC scatterplots and cell population locations



Combination of multiple plots and cluster analysis are used for **quantification** and **flagging** performance.

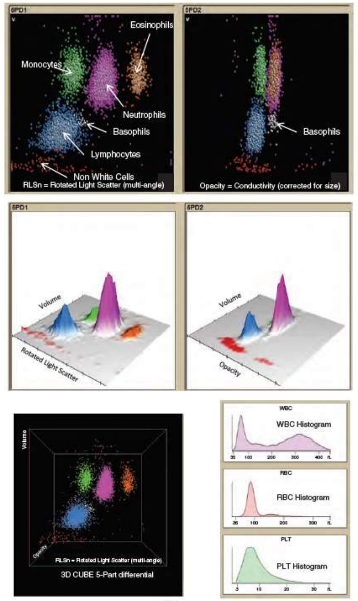
Source: Abbott

Impedance – Light Scattering (WBC) (Beckman)

VOLUME:
As opposed to using 0ø light loss to estimate cell size, VCS utilizes the Coulter Principle of (DC) Impedance to physically measure the volume that the entire cell displaces in an isotonic diluent. This method accurately sizes all cell types regardless of their orientation in the light path.

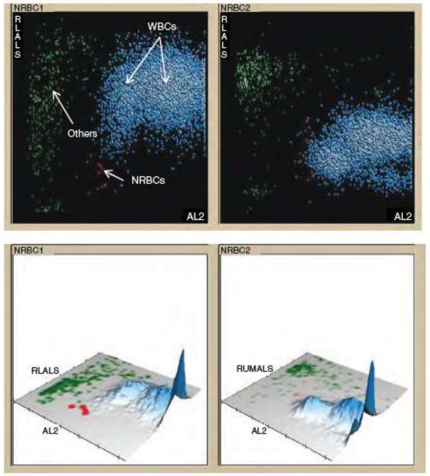
CONDUCTIVITY:
Alternating current in the radio frequency (RF) range short circuits the bipolar lipid layer of a cell's membrane, allowing the energy to penetrate the cell. This powerful probe is used to collect information about the internal structure of the cell, including chemical composition and nuclear volume.

SCATTER:
When a cell is struck by the coherent light of a LASER beam, the scattered light spreads out in all directions. Using a proprietary new detector, median angle light scatter signals are collected to obtain information about cellular granularity, nuclear lobularity and cell surface structure.



(a)

Fig. 2.9 Printouts from Beckman–Coulter DxH 800. (a) Scatter plots from the differential channel, five-part differential 1 (5PD1) and five-part differential 2 (5PD2), showing a plot of volume (v) against multi-angle rotated light scatter (RLSn) (left) and volume against opacity (right); in the corresponding three-dimensional representations (centre) the heights of the peaks reflect cell numbers; a com-



(b)

Fig. 2.9 *continued* (b) Two-dimensional and three-dimensional plots in the nucleated red blood cell (NRBC) channel showing the separation of NRBC from leucocytes; two light scatter measurements, RLAS (NRBC1, left) and RUMALS (NRBC2, right) are plotted against axial light loss (AL2), which measures the light absorbed as the cell passes through the flow cell (an indicator of cell size but also influenced by cellular transparency). By courtesy of Beckman–Coulter.

Blood cells, B Bain

Comparative performance

Table 2 Inter-instrument comparison of blood counts, reticulocyte and NRBC counts (n=349) and comparison of automated NRBCs or PLTs to microscopy or CD61 (n=30).

	System	τ_b	b	a	Mean	SD	95% limits of agreement
		Regression to median			Differences to median		
WBC, $10^3/\mu L$	Sapphire	0.98	1.00	0.00	−0.02	0.502	−0.70–0.60
	DxH 800	0.98	1.00	−0.00	0.03	0.309	−0.50–0.70
	Advia 2120i	0.97	**1.05**	−0.01	0.43	0.712	−0.17–1.70
	XE-5000	0.98	**0.98**	−0.02	−0.23	0.425	−1.00–0.08
	XN-2000	0.99	1.00	0.00	−0.05	0.187	−0.34–0.30
RBC, $10^6/\mu L$	Sapphire	0.98	1.00	0.00	0.01	0.037	−0.06–0.09
	DxH 800	0.97	**0.97**	−0.03	−0.13	0.049	−0.24 to −0.03
	Advia 2120i	0.96	1.00	**0.06**	0.06	0.062	−0.05–0.16
	XE-5000	0.97	1.00	0.00	0.01	0.087	−0.05–0.08
	XN-2000	0.96	**1.03**	−0.11	−0.02	0.078	−0.12–0.09
HGB, mmol/L	Sapphire	0.98	1.00	**0.10**	0.06	0.067	−0.06–0.19
	DxH 800	0.96	1.00	0.00	−0.03	0.113	−0.25–0.12
	Advia 2120i	0.98	1.00	0.00	0.01	0.070	−0.06–0.12
	XE-5000	0.96	1.00	**−0.10**	−0.07	0.249	−0.25–0.06
	XN-2000	0.98	1.00	0.00	−0.01	0.142	−0.12–0.12
HCT	Sapphire	0.96	1.00	0.00	−0.00	0.005	−0.01–0.01
	DxH 800	0.95	1.00	−0.01	−0.01	0.005	−0.02–0.00
	Advia 2120i	0.93	**1.04**	0.00	0.02	0.008	0.00–0.03
	XE-5000	0.96	1.00	−0.00	−0.00	0.008	−0.01–0.00
	XN-2000	0.96	**1.02**	−0.00	0.00	0.007	−0.01–0.01
MCV, fL	Sapphire	0.90	1.00	−1.20	−1.26	0.981	−3.30–0.00
	DxH 800	0.91	1.00	0.00	0.52	1.074	−1.50–3.00
	Advia 2120i	0.90	**1.08**	**−4.54**	2.35	1.145	0.20–5.00
	XE-5000	0.89	0.99	−0.74	−1.86	1.180	−4.50–0.00
	XN-2000	0.90	1.00	0.00	0.45	1.199	−2.40–3.10
MCH, pg	Sapphire	0.90	1.00	0.00	0.16	0.373	−0.40–1.10
	DxH 800	0.85	**1.10**	**−1.93**	0.95	0.588	0.00–2.10
	Advia 2120i	0.84	1.00	−0.50	−0.51	0.590	−1.40–0.40
	XE-5000	0.87	1.00	**−0.40**	−0.46	0.572	−1.50–0.20
	XN-2000	0.92	1.00	0.00	0.09	0.330	−0.50–0.80
PLT, $10^3/\mu L$	Sapphire	0.96	**1.07**	**−2.96**	3.44	7.840	−18.18–14.42
	DxH 800	0.96	**0.94**	−0.33	−5.05	8.219	−16.67–11.70
	Advia 2120i	0.95	**1.10**	−0.27	10.50	9.693	−0.78–29.63
	XE-5000	0.96	**0.97**	**1.31**	−1.32	9.834	−14.00–22.22
	XN-2000	0.97	1.00	0.00	−3.04	7.840	−25.00–7.89
RET, %	Sapphire	0.87	**1.19**	**0.05**	0.41	0.470	−0.10–1.20
	DxH 800	0.82	1.00	0.00	0.02	0.458	−0.60–1.30
	Advia 2120i	0.75	**0.86**	**−0.14**	−0.41	0.511	−1.80–0.30
	XE-5000	0.95	1.00	0.00	−0.04	0.152	−0.40–0.20
	XN-2000	0.91	1.00	0.00	0.07	0.196	−0.30–0.50
NRBC, %	Sapphire	0.57			−0.04	1.759	−0.80–1.00
	DxH 800	0.46			−0.09	1.752	−1.10–0.60
	Advia 2120i	0.47			0.27	5.636	−1.70–3.70
	XE-5000	0.85			0.24	1.768	0.00–1.40
	XN-2000	0.84			0.09	0.692	0.00–0.50
		Regression to microscopy			Differences to microscopy		
NRBC, %	Sapphire	0.54			−0.05	3.845	−2.00–1.10
	DxH 800	0.56			−0.23	3.481	−2.00–0.80
	Advia 2120i	0.37			0.26	6.062	−2.00–4.30
	XE-5000	0.63			0.20	3.297	−1.00–1.20
	XN-2000	0.66			0.03	2.701	−1.20–0.20
		Regression to CD61			Differences to CD61		
PLT, $10^3/\mu L$	Sapphire	0.92	1.04	0.21	7.72	22.95	−35.48–94.03
	DxH 800	0.91	**0.91**	**3.05**	23.84	47.24	−20.12–160.00
	Advia 2120i	0.93	**1.09**	**3.97**	42.07	43.09	−1.15–173.33
	XE-5000	0.90	1.01	**1.98**	19.75	33.60	−29.03–122.22
	XN-2000	0.96	0.97	0.59	2.24	17.86	−25.93–56.67

τ_b, Kendall's τ_b; b, slope (numbers in bold are significantly different from 1); a, intercept (numbers in bold are significantly different from 0); SD, standard deviation.

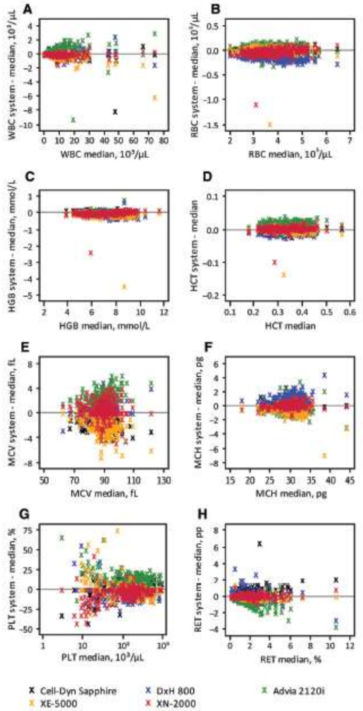


Figure 1 Difference plots for blood count parameters and reticulocyte counts.
Inter-instrument comparisons of blood and reticulocyte counts were determined in 349 routine samples. Differences between single measurements and the median of all five analyzers were plotted against the median of all analyzers. (A) WBC count, (B) RBC count, (C) hemoglobin concentration, (D) hematocrit, (E) MCV, (F) MCH, (G) PLT, (H) reticulocyte count.

DE GRUYTER Clin Chem Lab Med 2015; 53(7): 1057–1071

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Table 4 Inter-instrument comparison of pathological flaggings in 349 samples taken randomly out of routine analysis.

Instrument flagging	Pathological samples in microscopy, n	Instrument	True positives, n	Sensitivity 95% CI, %	False positives, n	Specificity 95% CI, %
Blasts	34	Sapphire	26	76 (59–89)	21	93 (90–96)
		DxH 800	25	74 (56–87)	15	95 (92–97)
		Advia 2120i	22	65 (46–80)	12	97 (94–98)
		XE-5000	22	65 (46–80)	6	98 (96–99)
		XN-2000	33	97 (85–100)	14	96 (93–98)
Variant lymphocytes	25	Sapphire	14	56 (35–76)	18	94 (91–97)
		DxH 800	16	64 (43–82)	18	94 (91–97)
		Advia 2120i	18	72 (51–88)	40	88 (84–91)
		XE-5000	20	80 (59–93)	17	95 (92–97)
		XN-2000	20	80 (59–93)	14	95 (93–98)
Immature granulocytes	90	Sapphire	49	54 (44–64)	24	91 (87–94)
		DxH 800	60	67 (56–76)	16	94 (90–96)
		Advia 2120i	35	39 (29–50)	11	96 (93–98)
		XE-5000	72	80 (70–88)	21	92 (88–95)
		XN-2000	82	91 (83–96)	35	86 (82–90)
Left shift	76	Sapphire	39	51 (40–63)	13	95 (92–97)
		DxH 800	64	84 (74–92)	27	90 (86–93)
		Advia 2120i	39	51 (40–63)	14	95 (92–97)
		XE-5000	38	50 (38–62)	1	99 (98–100)
		XN-2000	36	47 (36–59)	7	97 (95–99)
Platelet clumps	7	Sapphire	4	57 (18–90)	8	98 (96–99)
		DxH 800	6	86 (42–100)	7	98 (96–99)
		Advia 2120i	4	57 (18–90)	6	98 (96–99)
		XE-5000	4	57 (18–90)	8	98 (96–99)
		XN-2000	4	57 (18–90)	4	99 (97–100)
Blasts and/or variant lymphocytes	57	Sapphire	42	74 (60–84)	16	95 (91–97)
		DxH 800	46	81 (68–90)	15	95 (92–97)
		Advia 2120i	44	77 (64–87)	18	94 (90–96)
		XE-5000	43	75 (62–86)	11	96 (93–98)
		XN-2000	55	96 (88–100)	18	94 (90–96)
Blasts and/or variant lymphocytes and/or immature granulocytes	103	Sapphire	70	68 (58–77)	29	88 (84–92)
		DxH 800	80	78 (68–85)	29	88 (84–92)
		Advia 2120i	66	64 (54–73)	26	89 (85–93)
		XE-5000	88	85 (77–92)	30	88 (83–92)
		XN-2000	101	98 (93–100)	54	78 (72–83)

CI, confidence interval; n, number.

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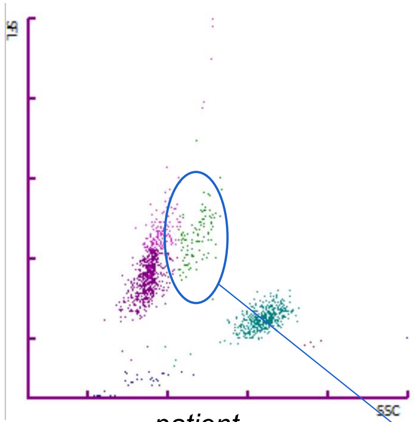
Conclusions

- **Multiple** techniques, each with their own **strengths and weakness**
- Quantification, differentation and flagging performance is based on “**behaviour**” of a cell population in a specific measuring method
- “Aspecific” measuring methods: systems designed to **count** normal cells and **detect** abnormalities, not for cell characterization (flow cytometry, cytomorphology)
- Scattergram/plots from an automated analyzer are an important source of information and may be an aid in interpretation for difficult cases.

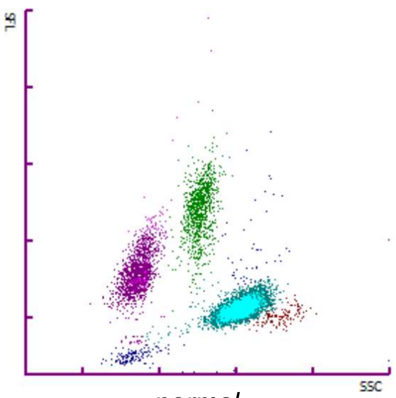
Example to illustrate the 'added value'

Male, 43yrs, referred from GP due to pancytopenia,
Recent travel: infectious? Acute leukemia? MDS?

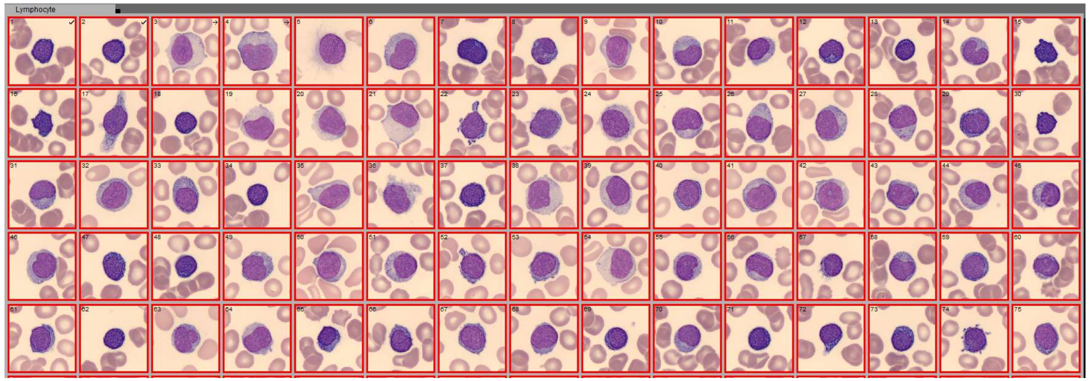
WBC	1.56	ſ	10*3/µL
RBC	3.97		10*6/µL
HGB	12.0		g/dL
HCT	36.8		%
MCV	92.7		fL
MCH	30.2		pg
MCHC	32.6		g/dL
PLT	46	&	10*3/µL
DIFF Profile			
NEUT#	540		/µL
LYMPH#	910		/µL
MONO#	110		/µL
EO#	0		/µL
BASO#	0		/µL
IG#	0		/µL
NEUT%	34.6		%
LYMPH%	58.3		%
MONO%	7.1		%
EO%	0.0		%
BASO%	0.0		%
IG%	0.0		%

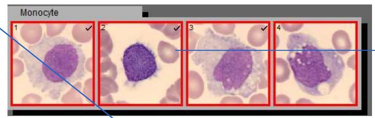


patient



normal

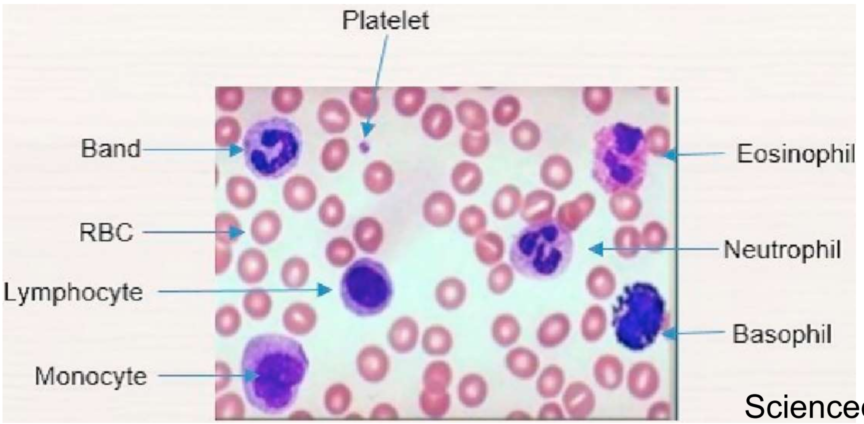




Only 1 'typical' Hairy Cell

Represent lymphocytes with high Ssc:
'signature' scattergram of HCL

Part 2: WBC-differentiation: Automated analyzer vs microscopy



Sciencedirect.com

Microscopy – golden standard?

- It's all about the number: Rümke table

Statistische imprecisie celdifferentiatie (naar Rümke)

Resultaat (%)	Aantal gedifferentiëerde cellen 100	200	500	1000	10000
	Verwacht resultaat				
0	0 - 3.6	0 - 1.8	0 - 0.7	0 - 0.4	0 - 0.1
1	0 - 5.4	0.1 - 3.6	0.3 - 2.3	0.5 - 1.8	0.8 - 1.3
2	0.2 - 7.0	0.6 - 5.0	1.0 - 3.6	1.2 - 3.1	1.7 - 2.3
3	0.6 - 8.5	1.1 - 6.4	1.7 - 4.9	2.0 - 4.3	2.6 - 3.4
4	1.1 - 9.9	1.7 - 7.7	2.5 - 6.1	2.9 - 5.4	3.6 - 4.5
5	1.6 - 11.3	2.4 - 9.0	3.3 - 7.3	3.7 - 6.5	4.5 - 5.5
6	2.2 - 12.6	3.1 - 10.2	4.1 - 8.5	4.6 - 7.7	5.5 - 6.5
7	2.9 - 13.9	3.9 - 11.5	4.9 - 9.6	5.5 - 8.8	6.5 - 7.6
8	3.5 - 15.2	4.6 - 12.7	5.8 - 10.7	6.4 - 9.9	7.4 - 8.6
9	4.2 - 16.4	5.4 - 13.9	6.6 - 11.9	7.3 - 10.9	8.4 - 9.6
10	4.9 - 17.6	6.2 - 15.0	7.5 - 13.0	8.2 - 12.0	9.4 - 10.7
15	8.6 - 23.5	10.4 - 20.7	12.0 - 18.4	12.8 - 17.4	14.3 - 15.8
20	12.7 - 29.2	14.7 - 26.2	16.6 - 23.8	17.6 - 22.6	19.2 - 20.8
25	16.9 - 34.7	19.2 - 31.6	21.3 - 29.0	22.3 - 27.8	24.1 - 25.9
30	21.2 - 40.0	23.7 - 36.9	26.0 - 34.2	27.2 - 32.9	29.1 - 31.0
35	25.7 - 45.2	28.4 - 42.0	30.8 - 39.4	32.0 - 38.0	34.0 - 36.0
40	30.3 - 50.3	33.2 - 47.1	35.7 - 44.4	36.9 - 43.1	39.0 - 41.0
45	35.0 - 55.3	38.0 - 52.2	40.6 - 49.5	41.9 - 48.1	44.0 - 46.0
50	39.8 - 60.2	42.9 - 57.1	45.5 - 54.5	46.9 - 53.1	49.0- 51.0
60	49.7 - 69.7	52.9 - 66.8	55.6 - 64.3	56.9 - 63.1	59.0 - 61.0
70	60.0 - 78.8	63.1 - 76.3	65.8 - 74.0	67.1 - 72.8	69.0 - 70.9
80	70.8 - 87.3	73.8 - 85.3	76.2 - 83.4	77.4 - 82.4	79.2 - 80.8
90	82.4 - 95.1	85.0 - 93.8	87.0 - 92.5	88.0 - 91.8	89.3 - 90.6
100	96.4 - 100	98.2 - 100	99.3 - 100	99.6 - 100	99.9 - 100

Rümke, C.L. The statistically expected variability in differential leukocyte counting. *In:* Koepke, J.A. (ed): Differential Leukocyte Counting. College of American Pathologists, Skokie, IL, 1978, p. 39.

98 × 558

-Aplasia samples ?!

Microscopy – golden standard?

- Cell-distribution on slide

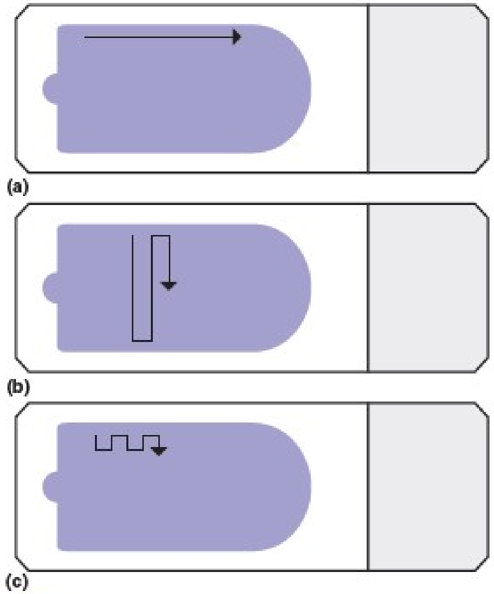
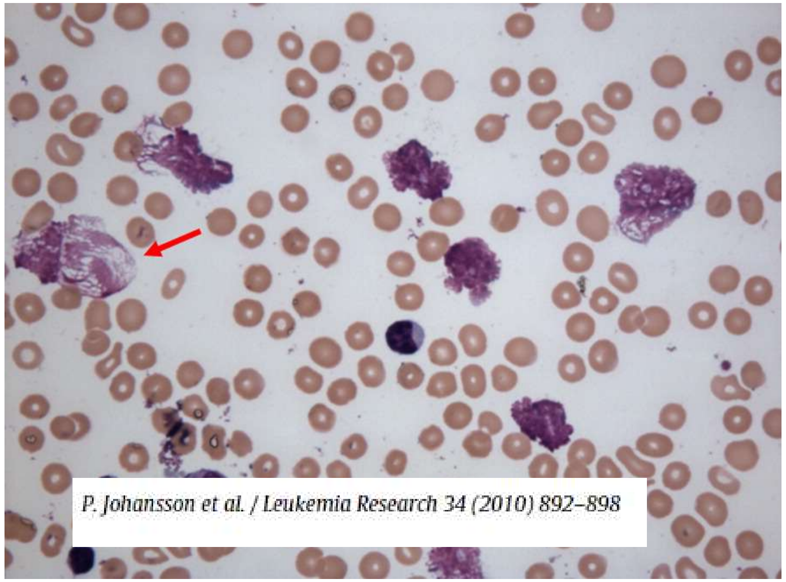


Fig. 2.3 Diagrams of blood films showing tracking patterns employed in a differential white blood cell count: (a) tracking along the length of the film; (b) battlement method; and (c) modified battlement method – two fields are counted close to the edge parallel to the edge of the film, then four fields at right angles, then two fields parallel to the edge and so on.

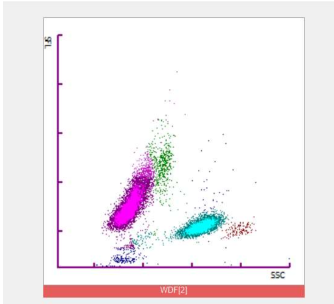
Microscopy – golden standard?

- Pre-analytical issues, eg smudge cells in CLL (and other lymphomas/reactive conditions)



P. Johansson et al. / Leukemia Research 34 (2010) 892–898

Analyzer diff



DIFF Profile			
NEUT#	2,480		/µL
LYMPH#	9,340		/µL
MONO#	1,770		/µL
EO#	50		/µL
BASO#	710		/µL
IG#	60		/µL
NEUT%	17.4		%
LYMPH%	65.1		%
MONO%	12.3		%
EO%	0.3		%
BASO%	4.9		%
IG%	0.4		%

Microscopy diff

BAND%			%
SEG%	51.4		%
LYMPH% (Diff)	35.5		%
MONO% (DIFF)	5.5		%
EO% (Diff)	2.2		%
BASO% (Diff)	1.1		%
VAR.LYMPH%	4.4		%
GIANTPL%	8.2		%
PLT CLUMPS%	7.1		%
ARTEFACT%	47.0		%
SMUDGE%	120.2		%
BAND#			/µL
SEG#	7,375.90		/µL
LYMPH# (DIFF)	5,094.25		/µL
MONO# (DIFF)	789.25		/µL
EO# (Diff)	315.70		/µL
BASO# (Diff)	157.85		/µL
VAR.LYMPH#	631.40		/µL

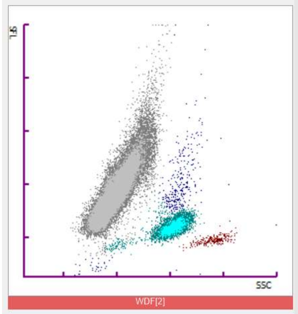
In most cases, **microscopy** is not the golden standard to **count** cell-populations

Microscopy vs Analyzer diff

- **Counting = Analyzer**

  except: -quantification of sub-populations that cannot be quantified by the analyzer (blasts, meta/myelo/promyelo,…)

  -populations cannot be clustered by the analyser



- **Screening** for and **characterization/confirmation** of **morphologic abnormalities = microscopy**

- Even **in the presence of abnormal cells**, it may be better to describe the morphology and to report the **analyzer diff** (prototype example, CLL)

Part 3: Workflow-organisation

Major “threat” in highly automated setting

- One tends to **loose control on individual samples** -> results are **reported** (and acted on) **before** results can be **reviewed** by the lab-supervisor

- Key to know and understand technical details, strengths and weaknesses, patient population, risk factors for spurious counts, … to implement **an optimal workflow** with minimal risk on clinically relevant errors.

- Process of **continuous** review, improvement and communication

Which samples need "review"?

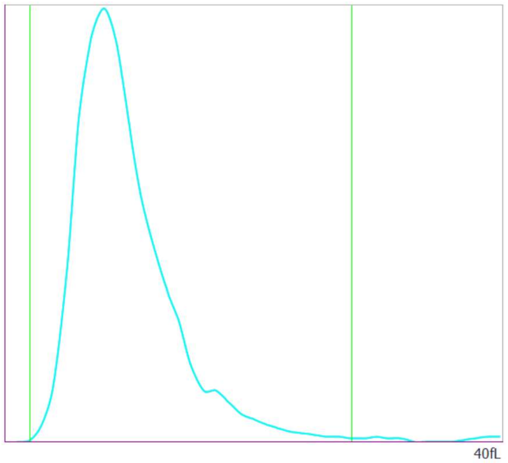
- Review: microscopy, scattergram review by technician/biologist, alternative methods,…

- Indications: -Screening for **abnormal cells**
  -WBC differentiation if **analyzer fails** to cluster
  -**explain observed flags and estimate impact**
  -exclude interferences

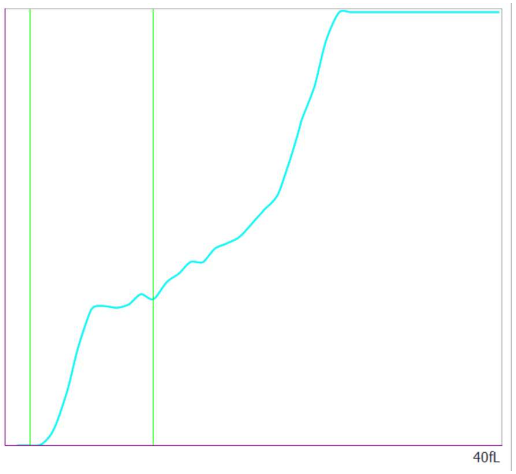
- Design of a rule set
  - **Technical** rules (ie reported results may not be reliable)
  - **"morphological"** rules (ie presence of abnormal WBC populations)
  - **"biological"** rules (ie unexpected or abnormal results-> close the gap in technical and morphological rules)

Technical rules (analyzer specific)

- PLT Abnormal distribution



normal



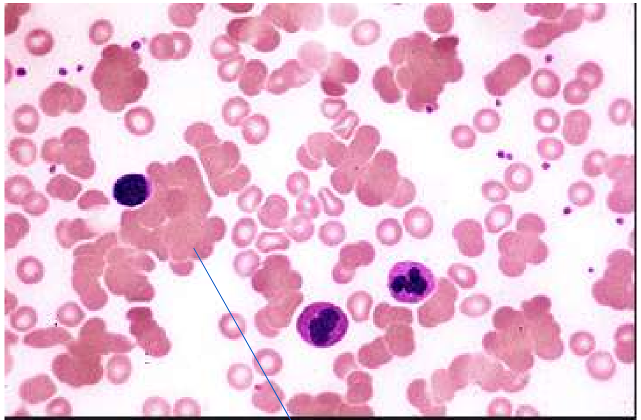
Presence of RBC-fragments

Reflex with another method/review of plausibility necessary

Technical rules (fabrikant-specifiek)

- Increased MCHC (1) (or discrepancy measured MCHC vs calculated MCHC)

Test	Run 1 - XN-1
SMEAR...	16/12/2020 12:21
Smear	
SMEAR...	DIFF
CBC Pro...	
WBC	10.24
RBC	0.54
HGB	9.6
HCT	6.5 (Bellen)
MCV	120.4
MCH	177.8
MCHC	147.7
PLT	194



Impedance: will be counted as 1 event

After incuation at 37°C

Run 6 -
16/1...
DIFF
12.73
2.78
9.5
28.2
101.4
34.2
33.7
217

Hb reliable, RBC not

Technical rules (fabrikant-specifiek)

- Increased MCHC (1) (or discrepancy measured MCHC vs calculated MCHC)

Test	Used	Run 2 - XN (37°C)	Run 1 - XN (KT)
CBC Pro...	07/09/20...	07/09/2020 17:18	07/09/2020 16:31
WBC	10.60	10.60	10.47
RBC	4.12	4.12	4.12
HGB	17.0	17.0	17.0
HCT	29.9	29.9	29.9
MCV	72.6	72.6	72.6
MCH	41.3	41.3	41.3
MCHC	56.9	56.9	56.9
PLT	338	338	351
RDW-CV	13.5	13.5	13.5
RDW-SD	35.2	35.2	34.6
MPV	12.2	12.2	12.6
P-LCR	44.3	44.3	45.6
PDW	17.3	17.3	18.1
PCT	0.41	0.41	0.44
STAAL			
DIFF Pr...			
NEUT#	4,940 (&)	4,940 (&)	4,990 (&)
LYMPH#	3,660	3,660	3,650
MONO#	1,710	1,710	1,530
EO#	50	50	40
BASO#	80	80	90
IG#	160	160	170

Test	Used	Run 2 - XN	Run 1 - XN
TNC-P	10.40	10.40	
RET Res...			
RBC-O	3.97	3.97	3.93
FRC#	0.0156	0.0156	0.0164
FRC%	0.38	0.38	0.40
RBC-HE	26.1	26.1	25.9
Delta-He	-0.2	-0.2	-0.4
RET-Y	156.5	156.5	155.1
RET-RB...	157.1	157.1	156.5
IRF-Y	149.4	149.4	148.2
RPI	0.4	0.4	0.4
HYPO-He	0.6	0.6	0.9
HYPER-...	0.2	0.2	0.3
RET-UPP	4	4	3
RET-TNC	114	114	97
LFR_RE...	88.1	88.1	89.3
MFR_R...	10.9	10.9	9.2
HFR_RE...	1.0	1.0	1.5
PLT-O	329	329	343
HGB-O	10.4	10.4	10.2
MCHC-O	34.8	34.8	34.1
Delta-HGB	6.6	6.6	6.8
Other Te...			

- Lipemia interference
- RBC ok, Hgb not

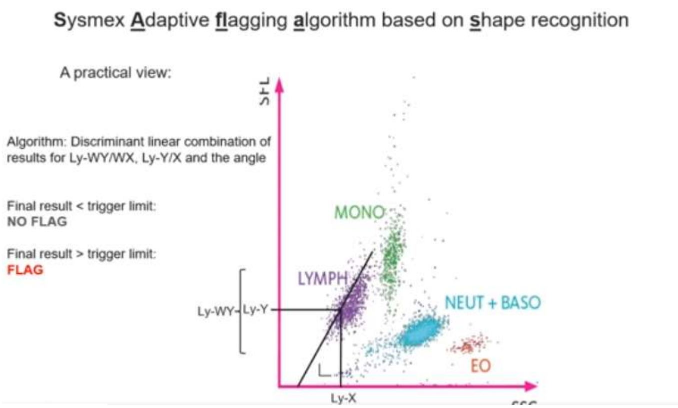
“Morphological” rules

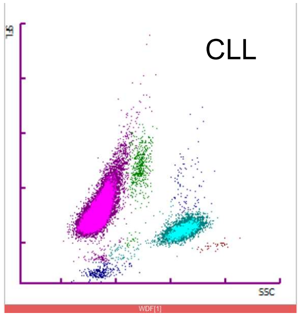
- WBC subpopulation behave differently compared to normale samples (higher RNA content, more/less granularity, larger cells,…) => Requires **microscopy review**

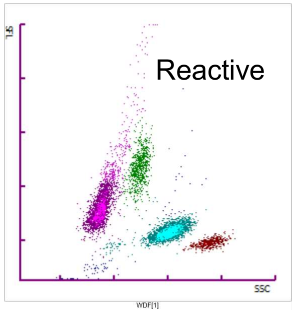
- Population specific exceptions are possible:
  - No differentiation of Immature Granulocytes
  - Patient known with normoblasts -> no confirmation/screening
  - Known CLL-patients -> report analyzer diff/confirm morphology
  - …

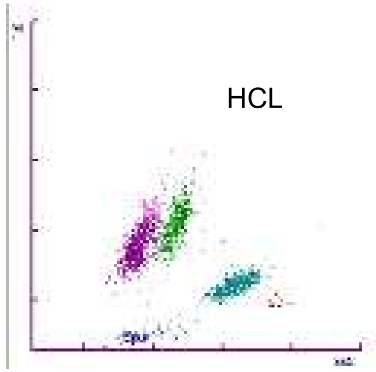
“Morphological” rules

- Blast/Abn Lymph









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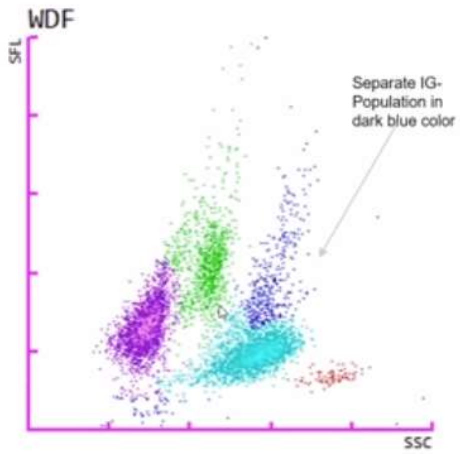


- …

Biological rules

Smear microscopy revision: propositions by the GFHC

F. GENEVIÈVE[1], A.C. GALOISY[2], D. MERCIER-BATAILLE[3], O. WAGNER-BALLON[4], F. TRIMOREAU[5], O. FENNETEAU[6], F. SCHILLINGER[7], V. LEYMARIE[5,8], S. GIRARD[9], C. SETTEGRANA[10], S. DALIPHARD[11], V. SOENEN-CORNU[12], M. CIVIDIN[13], J.F. LESESVE[14], B. CHÂTELAIN[15], X. TROUSSARD[16], V. BARDET[17]
for the Francophone Group of Cell Haematology

ABOUT THE ISLH

Consensus Guidelines: Preface

The International Consensus Group for Hematology Review is pleased to publish the attached guideline:

Suggested Criteria for Action Following Automated CBC and WBC Differential Analysis

Biological rules

- Based on **quantitative abormalities**

Table 3: Indications for smear review in terms of the results of the WBC differential.

Former result	Adults/children	Presence of malignant cells, as observed with the former result Presence of NRBC, as observed with the former result (if they are not counted automatically by an analyser)
NRBC	Adults/children	NRBC have been detected by the analyser, in an initial situation or every time if they are not counted automatically by the analyser
Neutrophils	Adults/children	$< 1.5 \times 10^9$ cells/L, in an initial situation
Eosinophils	Adults/children	$> 1.5 \times 10^9$ cells/L, in an initial situation
Basophils	Adults/children	$> 0.3 \times 10^9$ cells/L and/or > 3%, in an initial situation
Lymphocytes	Adults	$> 5 \times 10^9$ cells/L, in an initial situation
	Children	$> 9 \times 10^9$ cells/L (two to six years), $> 6 \times 10^9$ cells/L (six to 12 years), $> 4 \times 10^9$ cells/L (> 12 years), in an initial situation
Monocytes	Adults/children	$> 1.5 \times 10^9$ cells/L, in an initial situation $> 1.5 \times 10^9$ cells/L, if persistent for more than 30 days > a threshold, which is to be defined for each laboratory when monocytosis occurs during hospitalisation

Biological rules

- Based on **patient characteristics**

3.1.1. Is it necessary to do a smear systematically depending on the age of the patient?

A patient's age is not a criterion for adults. With neonates, during the first week of life, smear revision is recommended at least at the time the first CBC is performed, due to the frequent erythroblastaemia (see also the section 'Indications regarding the WBC diff'). In children younger

3.1.2. Prescribing physician or hospitalisation service

A systematic smear review is needed for patients from the paediatric haematology-oncology unit that are unknown or without recent morphological information. This is due mainly to the fact that analysers usually have problems detecting lymphoblast cells when they are present in low numbers (18). Apart from this particular situation, a physician's opinion is not considered a criterion that must lead to a smear review. The biologist in the lab can trust

3.1.3. Permanent reference regarding information of the patient

If an abnormality was identified for the first time in a patient, registering a permanent comment associated with that patient's information can be useful for validating subsequent CBCs faster and more securely. An example would be the presence of cryoglobulins or WBC agglutinations, which are important in terms of the cell count. A permanent message associated with the patient that points out this situation can be used as a criterion for performing the analysis at 37°C or a smear review next time.

3.1.4. Specific prescription of the morphological analysis

This type of prescription necessarily involves smear review and an explicit comment to the prescribing physician in return. In the absence of abnormal cells, the analyser cell count, which is more precise, is preferred to the manual count. If the prescription asks for schizocytes, the search for them can be performed differently. The responsible biologist can decide whether or not there is a need to perform a blood smear. This will depend on the laboratory and whether its analyser is capable of quantifying RBC fragments (19). If a schizocytes count is required in the end, this will be done in line with the recommendations published recently (20).

Diagnostic PB-sample of AML-M3 (hypoleukocytair)

Rules

57. Multiple runs!

WBC morph positive -> Smear!

71. Leukocytopenia in "Initial situation" -> Smear

50. HgB < 7 -> INFORM DOCTOR

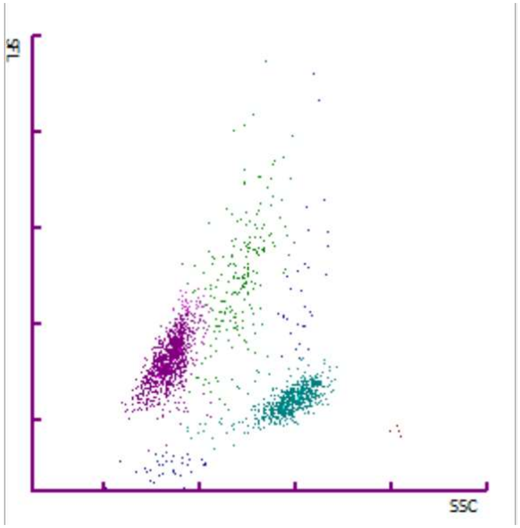
76. WBC < 2.5 -> INFORM DOCTOR

110. PLT low in "initial situation" -> CHECK FOR CLOT and check smear

114. Neutropenia in "Initial situation" -> Smear

'biological' rules

STOLSEL-INFO	Geen stolsel		
WBC	2.27	Bellen	10*3/µL
RBC	2.62		10*6/µL
HGB	6.9	Bellen	g/dL
HCT	20.7		%
MCV	79.0		fL
MCH	26.3		pg
MCHC	33.3		g/dL
PLT	21		10*3/µL
RDW-CV	15.0		%
RDW-SD	41.5		fL



3% promyelo/blasts -> no 'morphological' rules

Conclusions

- Current **automated hematology systems** are gold standard **to count and differentiate** blood cells for the majority of samples
- **Understand the technical principles** to resolve **spurious counts and** to detect **abnormal cells**
- A well designed rule set is key to optimize the process of automated counting and differentiation

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