

HKMB Tečaj trajne edukacije: Osnove laboratorijske hematologije, 7. prosinca 2024.

# Specifikacije hematoloških analizatora

Brankica Šimac

Klinički zavod za laboratorijsku dijagnostiku

KLINIČKA  
BOLNICA  
DUBRAVA

Differential methods used:	Interfering substances:	Min. specimen volume open mode/Closed mode/Microsample capability:	≤100 µL/≤100 µL	LIS interface formats supported:	ASTM 1394-91, ASTM 1381, HL7 version 2
Analytical measurement range:	• WBC/RBC:	Types of patient data archived/Max. No. of archived patient data accessible when analyzer online:	• Reagents ready to use/Reagent tracking method:	• Middleware interface:	yes (can use company's middleware or third directly to LIS)
• WBC count/RBC count:	• Hemoglobin/Platelets:	Analyzer performs delta checks:	Time required for analyzer maintenance:	• Information transferred to LIS via middleware or LIS interface:	numeric results, flagged results, histogram demographics, patient orders, more
• Hemoglobin/Platelets:	• Hematocrit/Reticulocytes:	Parameters for which flags may appear:	Onboard diagnostics for troubleshooting:	Analyzer transmits LOINC codes for results to LIS or middleware:	no
• Hematocrit/Reticulocytes:	• Automated differential:	• Flagging client selectable:	• Manufacturer can perform diagnostics via remote access:	Lab automation system or workcells to which analyzer has a direct interface:	Abbott Diagnostics
Precision:	Throughput: max. CBCs per hour/Max. CBCs with differentials per hour:	Analyzer recognizes and holds test results:	Warranty provided/Cost of annual service contract (24 h/7 d):	Barcode symbologies read on specimen tube:	Codabar, Code 39, Code 128, Interleaved 2
• WBC count/RBC count:	• Max. No. of slides processed per hour if stainer included:	Scattergram display uses cell-specific color:	Distinguishing analyzer features (supplied by company):	• Analyzer allows barcode placement per CLSI standard Auto02-A2:	yes
• Hemoglobin/Platelets:		Histogram display uses color with thresholds:	yes		
• Hematocrit/Reticulocytes:					

**Kako na temelju specifikacija  
odabrati hematološki  
analizator?**



**Razumijevanje metoda rada hematoloških analizatora**

**Vrsta medicinsko-biokemijskog laboratorija**

**Budžet medicinsko-biokemijskog laboratorija**

**Marketinški pristup proizvođača**

**Servisna podrška**



# Povijesni pregled razvoja hematoloških analizatora



Wallace H. Coulter  
patentirao Coulterov princip

**1953**

**1956**

Prvi hematološki brojač  
Coulter model A

**1968**

Prvi višeparametarski hematološki  
brojač Coulter model S  
(L,E, Hb, Htc, MCV, MCH, MCHC)

**1974**

Prvi automatizirani 5-diff  
analizator Technicon  
HEMLOG D

**1977-1986**

Prvi hematološki analizator koji  
uključuje i Trc, RDW, MPV i 3 diff  
(Coulter Model S+, S+II, S+IV,  
Ortho ELT-8)

**1985**

Prvi automatizirani CBC i  
6-diff analizator  
Technicon H\*1

**1996**

Uvedena metoda fluorescencije

**1998**

Razvoj i modificiranje  
postojeći metoda od  
strane različitih  
proizvođača

# GLOBALNO TRŽIŠTE HEMATOLOŠKIH ANALIZATORA

Ukupni tržišni udio 2024.:

4,44 milijardi EUR

Sjeverna Amerika i Kanada 41,3%

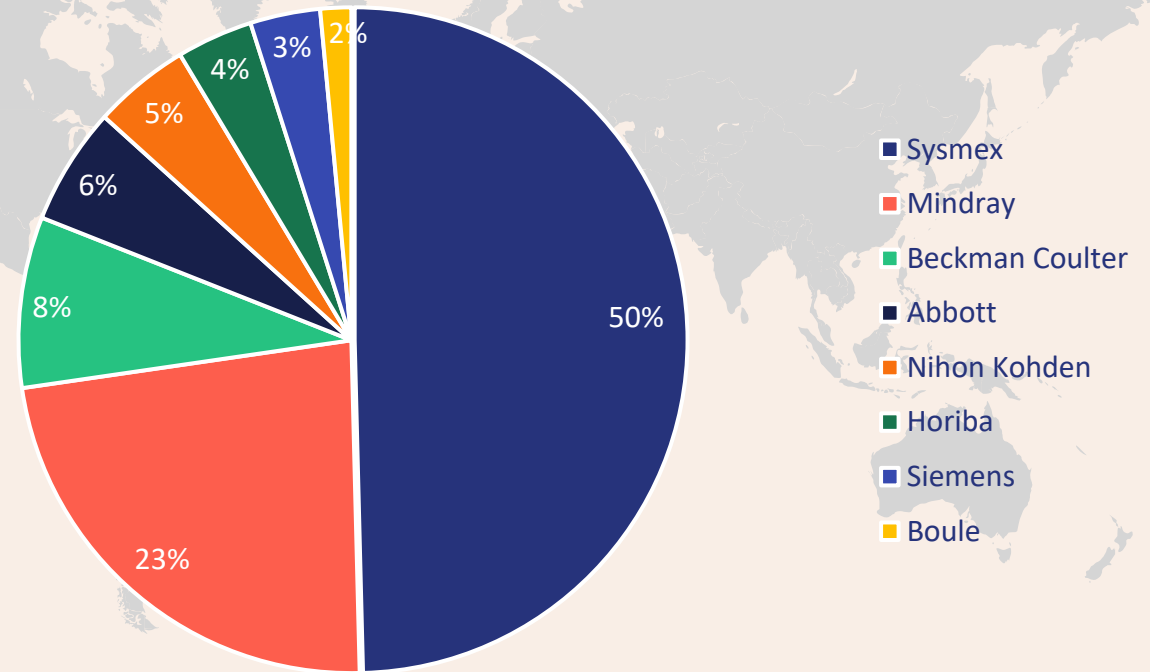
Procjena rasta 2024.-2031.: 7,3%

Očekivani najveći rast: zemlje  
jugoistočne Azije

Procjena tržišnog udjela 2031.:

8,35 milijardi EUR

Tržišni udio prema proizvođaču 2024.\*



\*temeljeno na izvješćima organizatora VKK Riqaş, uključuje >6300 analizatora iz laboratorija diljem svijeta, različiti modeli (3-diff, 5-diff, 6-diff, automatizirani, polu-automatizirani)

Automatizirani hematološki analizatori: 62,8%

Dijagnostički laboratoriji: 41,6%

## Metode rada hematoloških analizatora:

1. **Metoda impedancije**
2. **Metoda radiofrekvencije**
3. **Optička metoda**  
Flouorescencija  
Citokemija



# Metoda impedancije

Krvne stanice su loši električni vodiči.

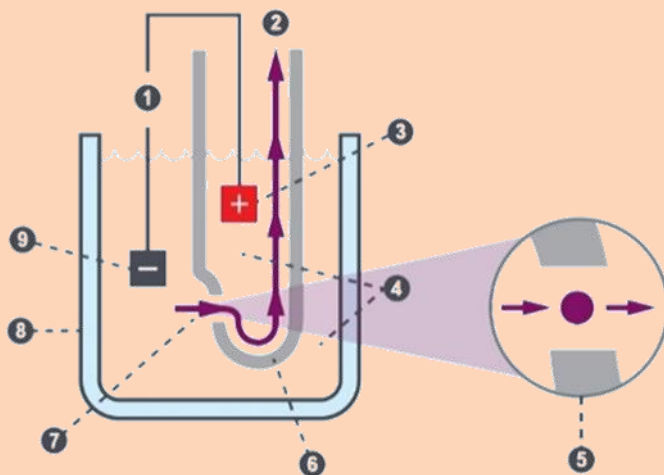
Krvne stanice razrjeđene u otopini elektrolita koja služi kao vodič istosmjerne električne struje između dvije elektrode koje nadziru elektronski senzori.

Prolazak krvnih stanica kroz otvor između elektroda uzrokuje promjenu električnog otpora i pojavu mjerljivih promjena napona u obliku pulsno vala.

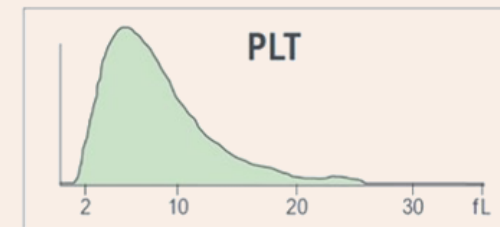
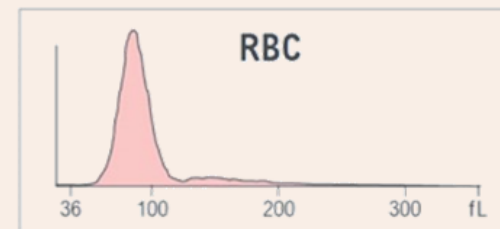
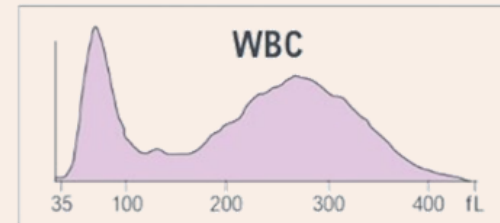
Broj nastalih promjena u naponu razmjern je broju stanica.

Veličina promjene napona, odnosno amplituda ili intenzitet svakog pulsno vala, izravno je razmjerna volumenu stanica.

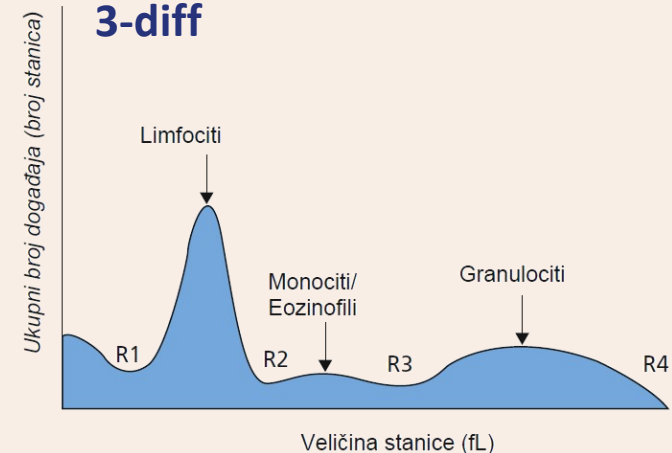
- 1 Struja otvora
- 2 Vakuum
- 3 Interna elektroda
- 4 Suspenzija krvnih stanica
- 5 Detalj otvora
- 6 Cijev otvora
- 7 Otvor (apertura)
- 8 Komorica
- 9 Vanjska elektroda



## Histogrami



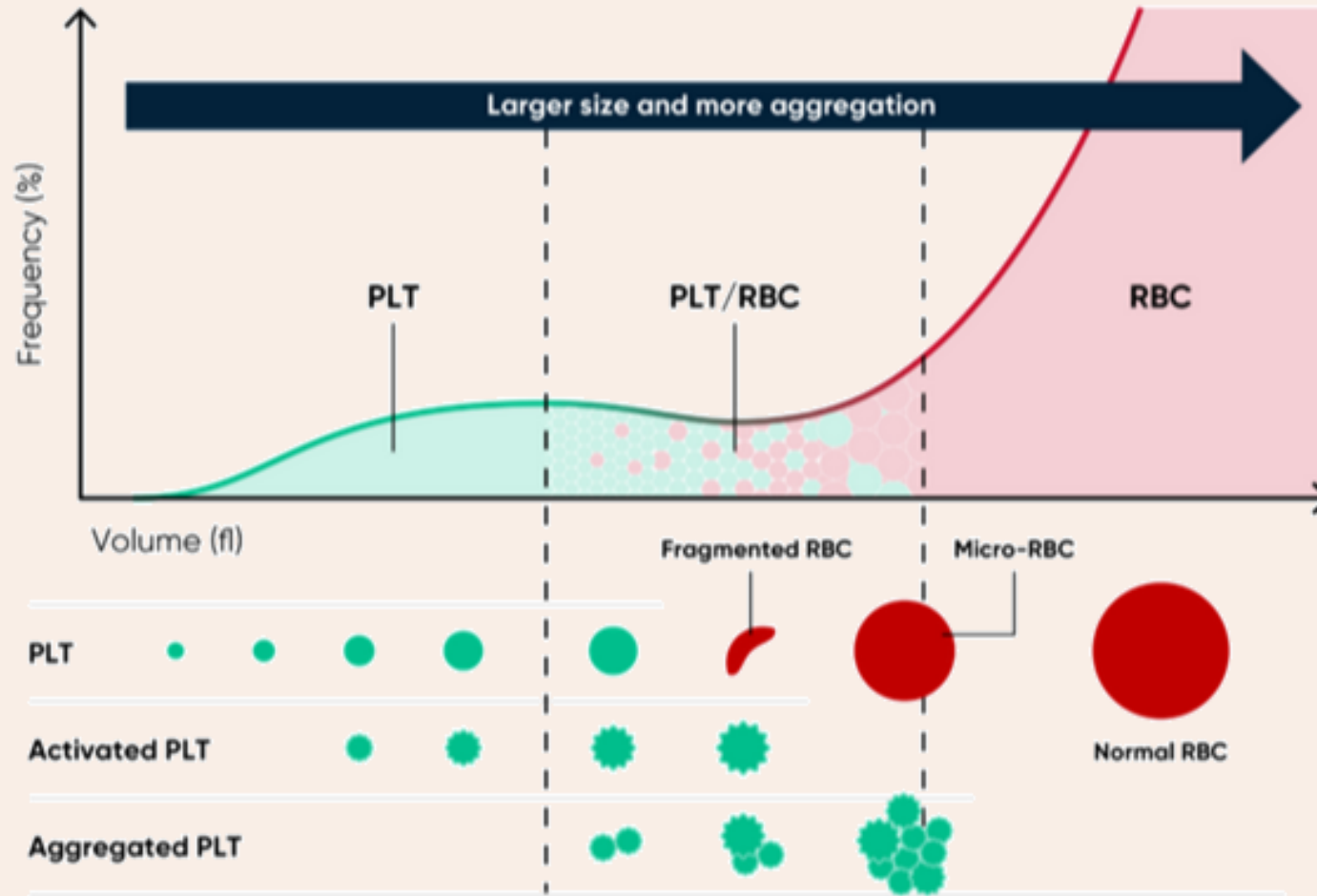
## 3-diff





# Metoda impedancije

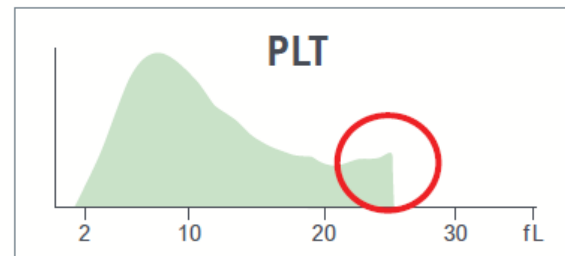
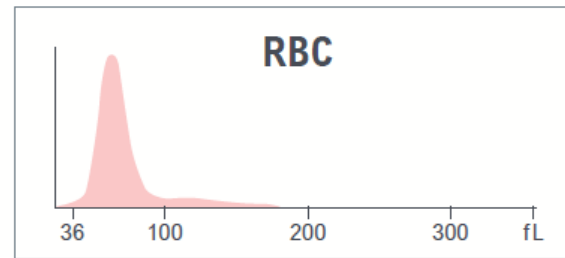
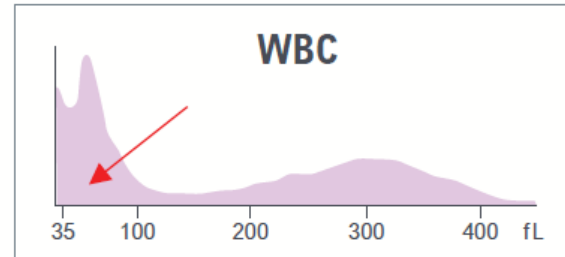
## Ograničenje metode - Interferencije



# Metoda impedancije

## Interferencije

Test	Result	Flags
WBC	5.6	R
UWBC	8.3	R
RBC	4.50	
HGB	9.6	L
HCT	30.3	L
MCV	67.3	aL
MCH	21.4	L
MCHC	31.8	L
RDW	16.1	
RDW-SD	36.8	
PLT	83	R L
MPV	10.6	R



Suspect messages

- Giant Platelets

System messages

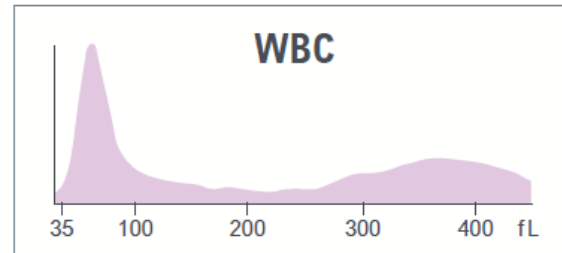
- Cellular Inter-Platelet Clumps

Nakupine trombocita

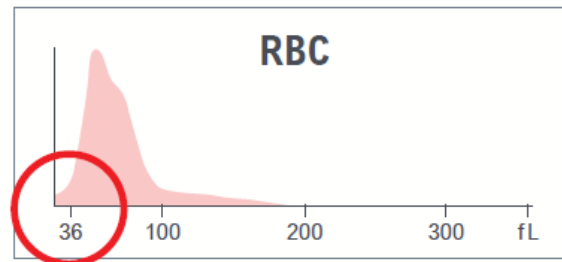
# Metoda impedancije

## Interferencije

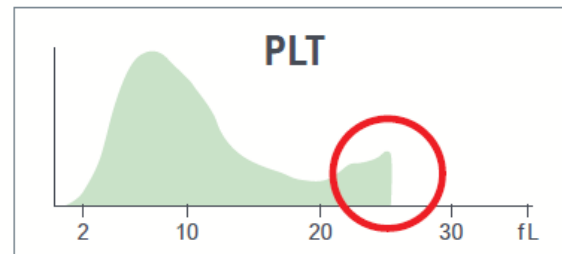
Test	Result	Flags
WBC	9.4	
UWBC	9.4	
RBC	5.09	
HGB	10.3	L
HCT	32.6	L
MCV	64.1	cL
MCH	20.2	L
MCHC	31.5	L
RDW	26.5	aH
RDW-SD	55.6	H
PLT	471	H
MPV	9.9	



System messages  
• Dimorphic Reds



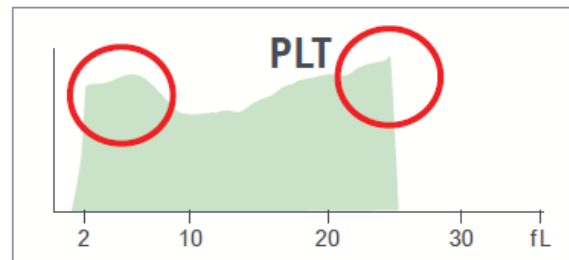
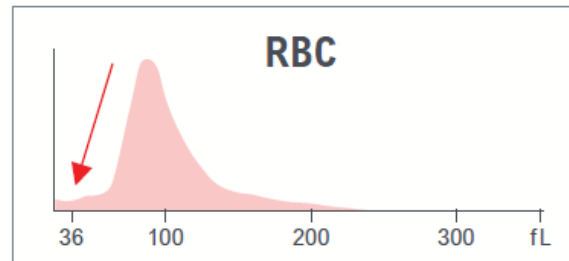
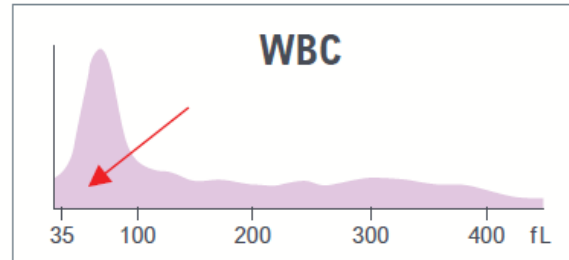
Dimorfni  
eritrociti



# Metoda impedancije

## Interferencije

Test	Result	Flags
WBC	8.3	
UWBC	8.3	
RBC	3.00	L
HGB	10.0	L
HCT	29.2	L
MCV	97.2	H
MCH	33.3	
MCHC	34.3	
RDW	22.7	aH
RDW-SD	74.8	H
PLT	27	RaL
MPV	6.5	R L

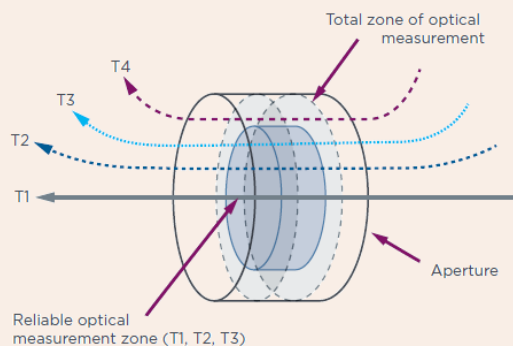


System messages

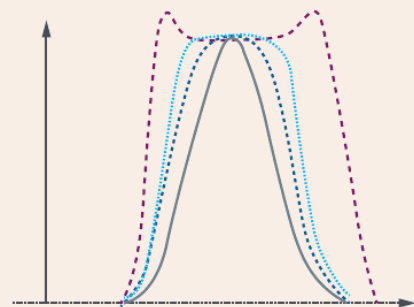
- PLT Inter: Debris
- RBC-PLT overlap

Fragmenti stanica

## Puls editing



T4  
T3  
T2  
T1



## Triple counting - voting

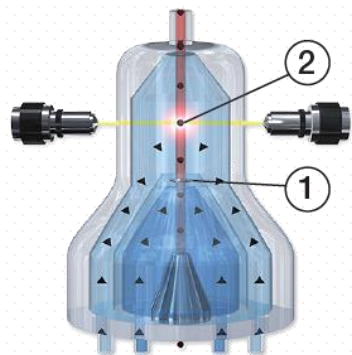
Figure 5. Triple Counting Data

		WBC	UWBC	RBC	MCV	RDW	PLT	MPV
APERTURE 1	<input checked="" type="checkbox"/>		4.059	4.617	94.65	13.06	153.5	10.14
APERTURE 2	<input checked="" type="checkbox"/>		3.916	4.643	93.75	13.06	155.7	10.15
APERTURE 3	<input checked="" type="checkbox"/>		4.042	4.610	93.61	13.17	163.0	9.99
	AVG	4.006	4.006	4.623	94.00	13.10	157.4	10.09

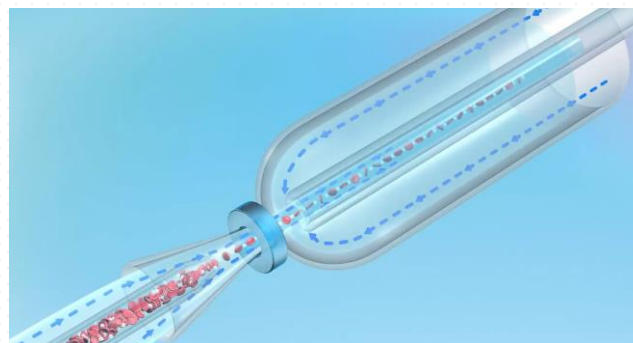
# Tehnička rješenja

## za prevladavanje ograničenja metode impedancije

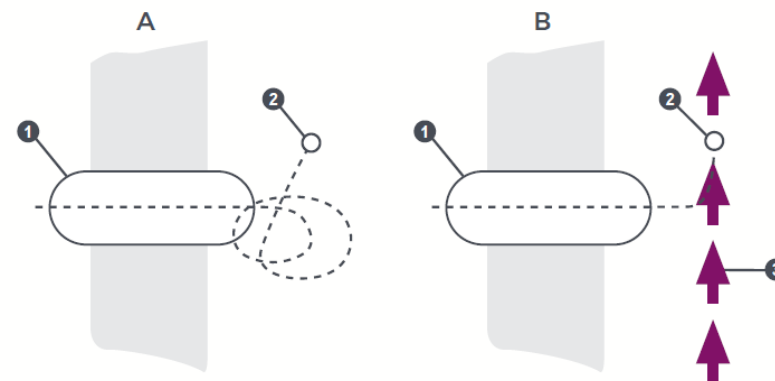
### Double Hydrodynamic Sequential System (DHSS) technology



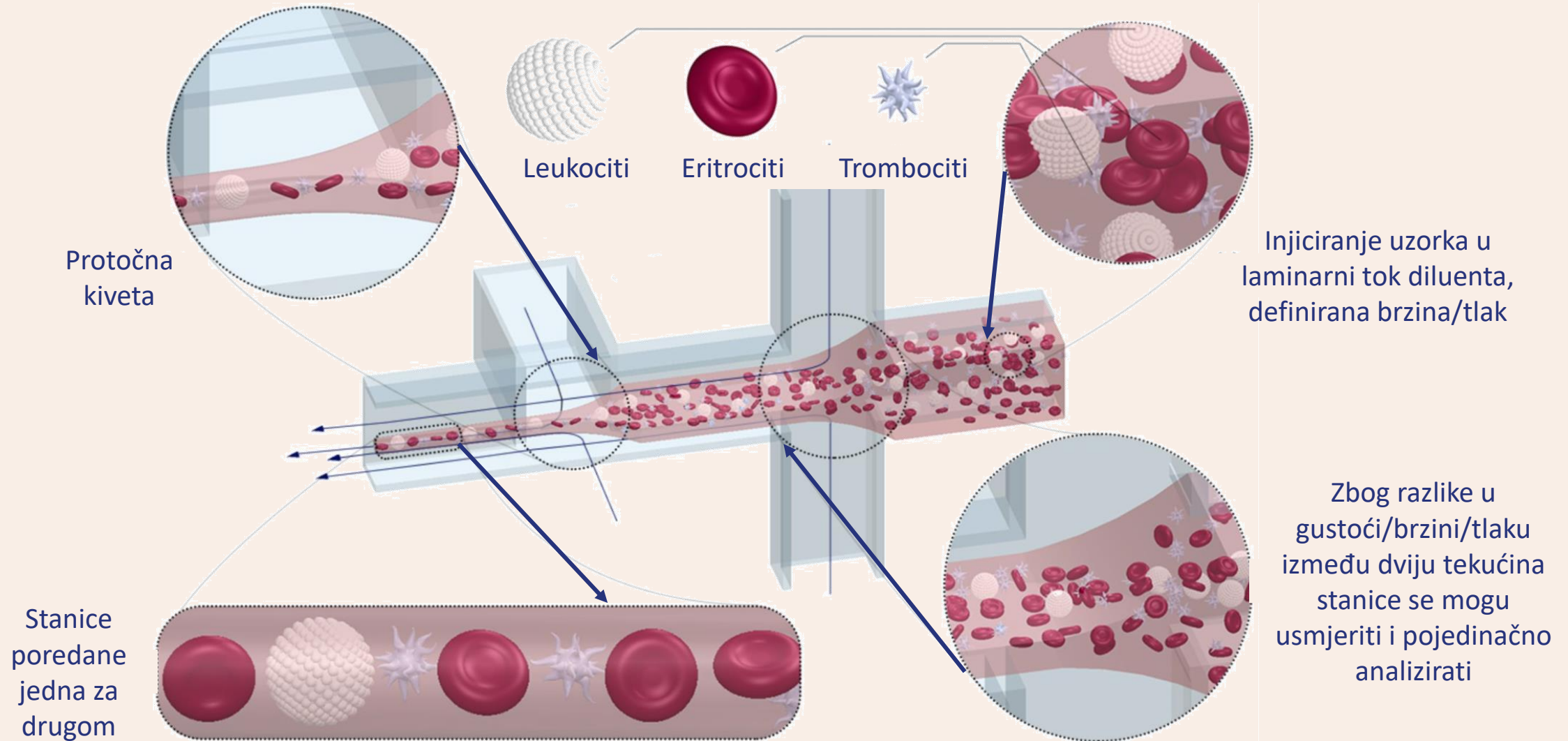
### DC Sheath Flow Detection



### Sweep Flow



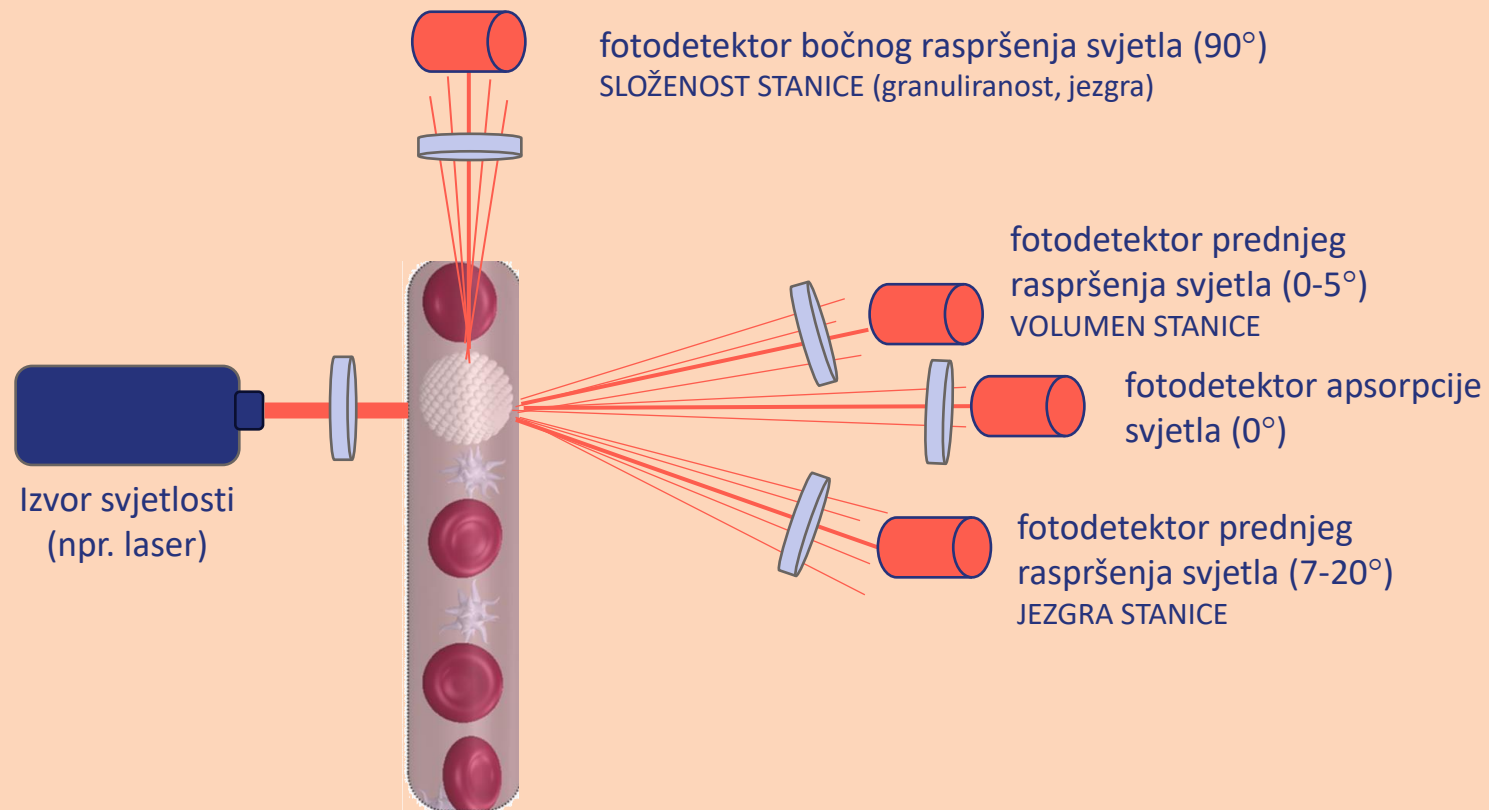
# Hidrodinamičko fokusiranje



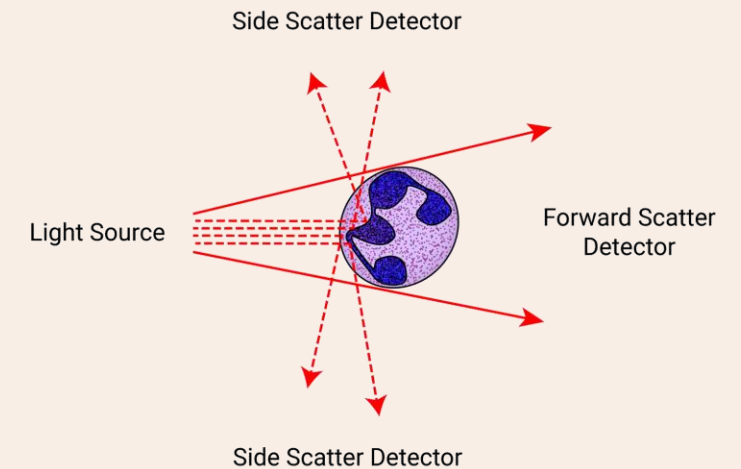
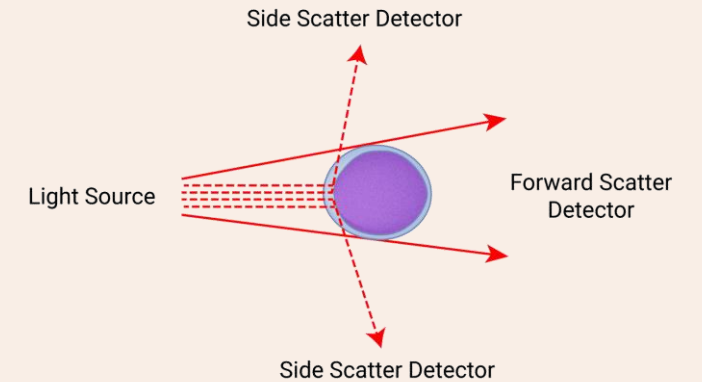
# Optička metoda

Temelji se na fizikalnim karakteristikama stanica.

Primjenjuje se za diferenciranje leukocita, određivanje broja stanica, detekciju specifičnih staničnih karakteristika.



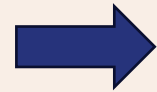
Stanice u usmjerenom toku prolaze kroz protočna kivetu:  
Hidrodinamičko fokusiranje



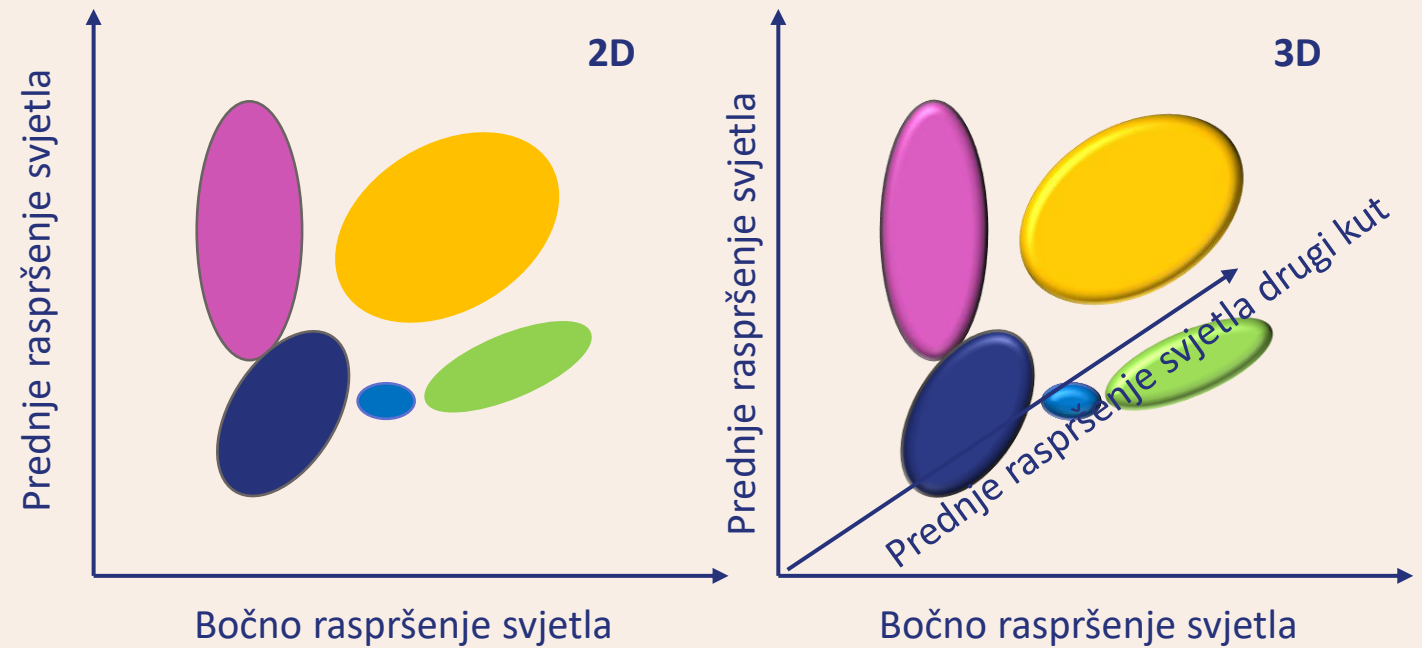


# Optička metoda

Fotostruja iz detektora se digitalizira i obrađuje u softveru analizatora

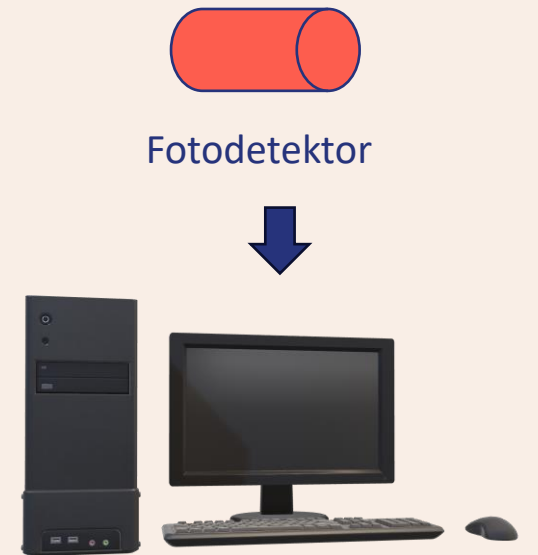
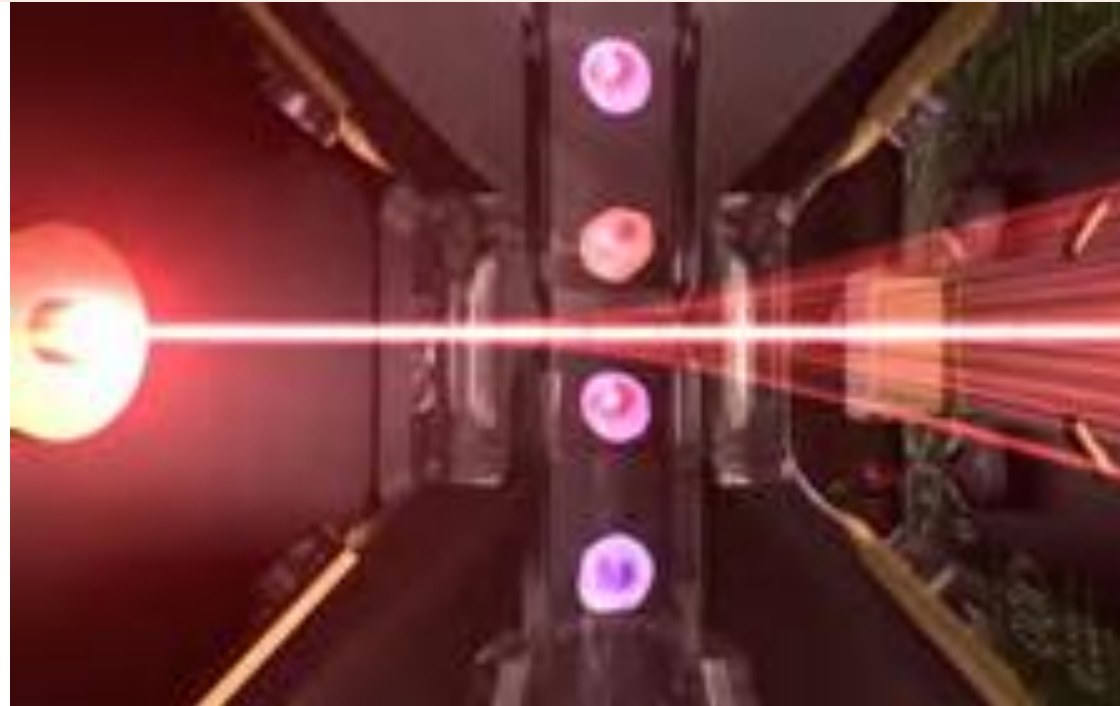
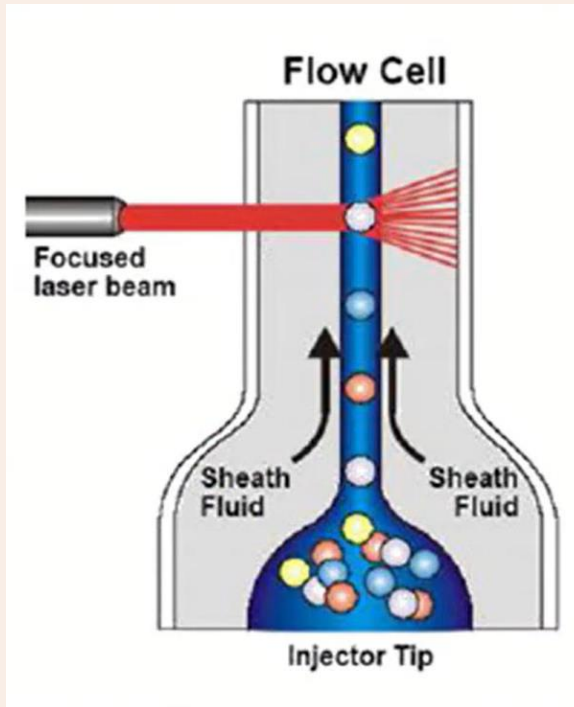


## Dijagrami raspršenja



Stanice sličnih fizikalnih karakteristika grupiraju se u skupine na temelju zadanih algoritama

# Protočna citometrija



HIDRODINAMIČKO FOKUSIRANJE

+

OPTIČKI SISTEM

+

ELEKTRONIKA

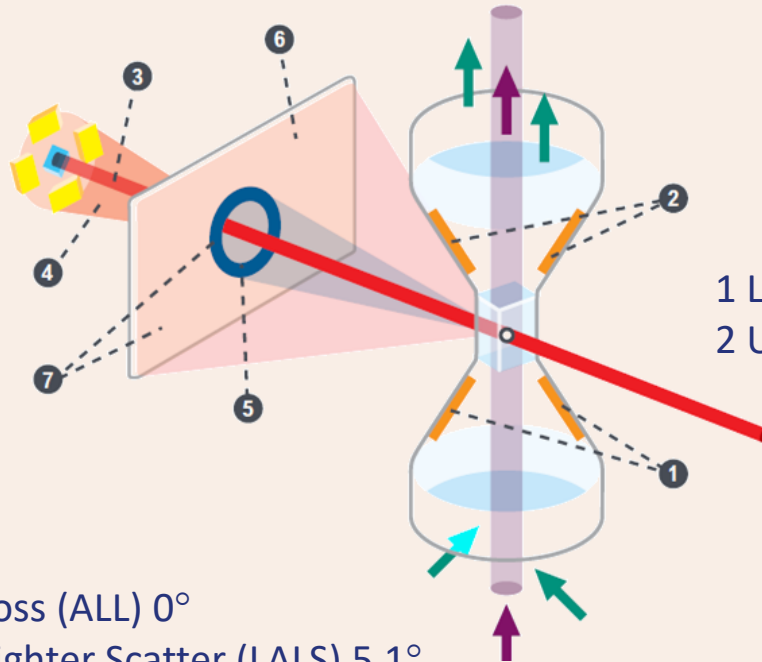
**Proizvođači primjenjuju  
različite pristupe za  
diferenciranje leukocita**

# Optička metoda

Beckman Coulter DxH 900/DxH 690T

VCS tehnologija za diferenciranje leukocita

Kombinira 3 metode: Impedancija – volumen (V); Radiofrekvencija – vodljivost (engl. conductivity - C); Optička – (light scatter – S)



1 Lower Electrode (DC/RF)  
2 Upper Electrode (DC/RF)

3 Axial Light Loss (ALL)  $0^\circ$

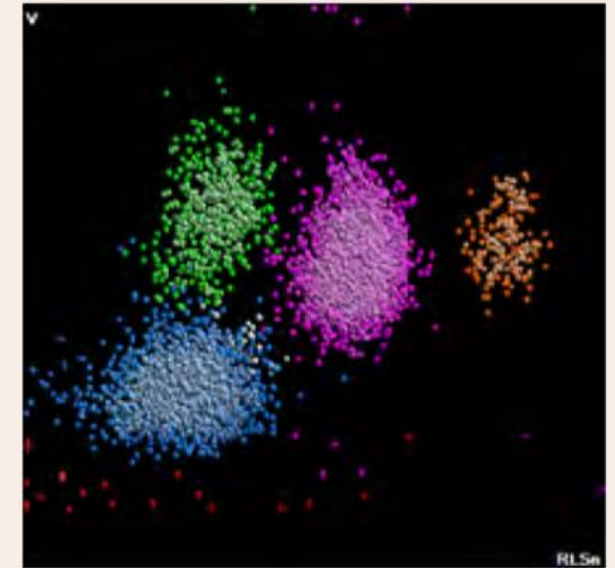
4 Low Angle Lighter Scatter (LALS)  $5.1^\circ$

5 Lower Median Angle Light Scatter (LMALS)  $10^\circ$ - $20^\circ$

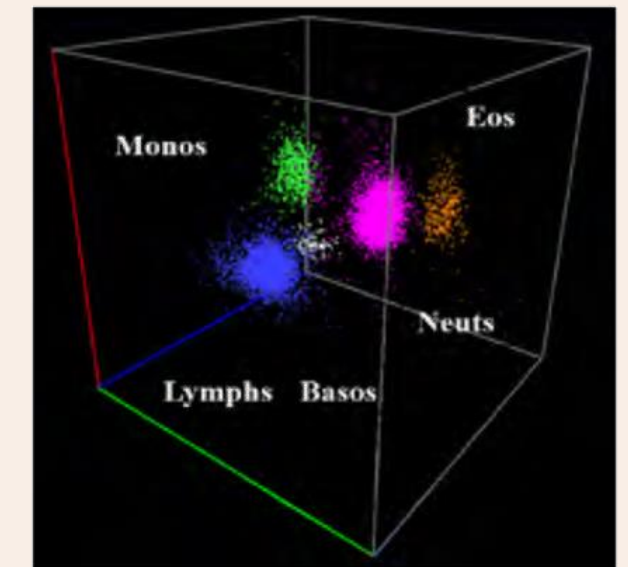
6 Upper Median Angle Light Scatter (UMALS)  $20^\circ$ - $42^\circ$

7 The fifth light scatter channel is the sum of the UMALS and the LMALS regions

2D Dijagram raspršenja



3D Dijagram raspršenja



# Metoda radiofrekvencije

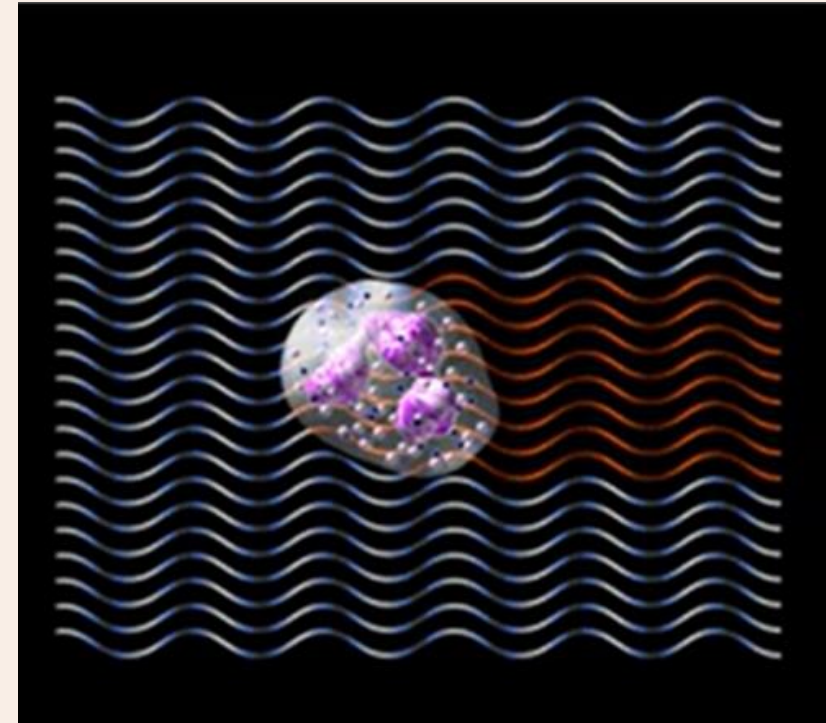
Koristi se za mjerenje staničnog sadržaja.

Primjenjuje se izmjenična struja koja proizvodi elektromagnetsko polje visoke frekvencije ili radiofrekvencije (RF).

Bipolarni lipidni sloj membrane stanice djeluje kao provodnik energije.

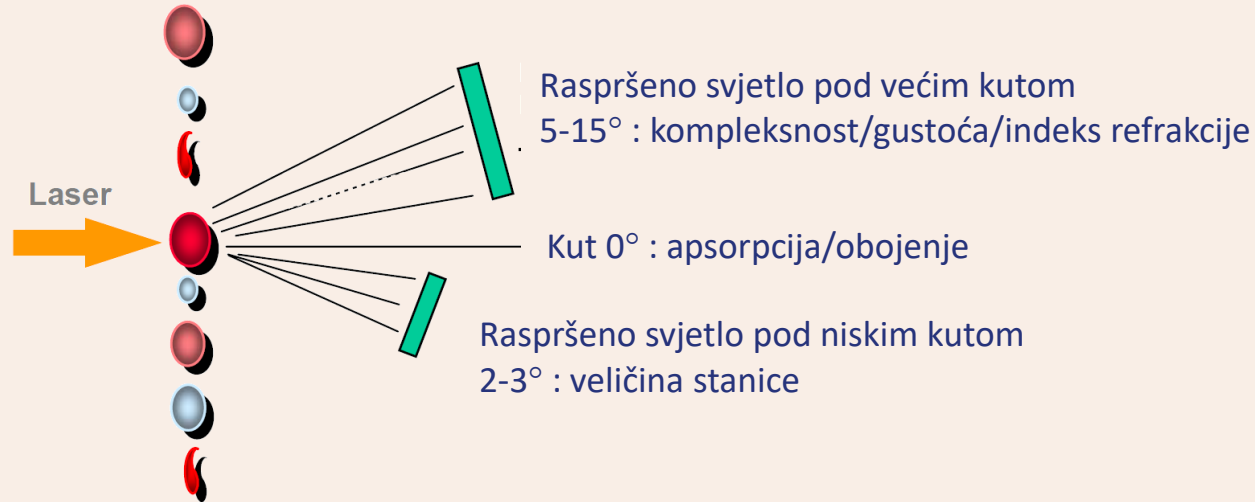
Detektira se promjena intenziteta RF ovisno o unutrašnjoj složenosti stanice kao što je granuliranost citoplazme, veličina, izgled i volumen jezgre i druge strukture.

Koristi se u kombinaciji s drugim metodama za diferencijaciju leukocita (kao npr. VCS tehnologija kod Coulter analizatora).



# Optička metoda + citokemijska reakcija

Siemens Advia 2120i



## Perox kanal

Leukociti se identificiraju na temelju veličine i prisutnosti / aktivnosti enzima mijeloperoksidaze (MPO). Dodatkom supstrata za MPO stanice se boje različitim intenzitetom, a detektira se intenzitet apsorpcije svjetla.

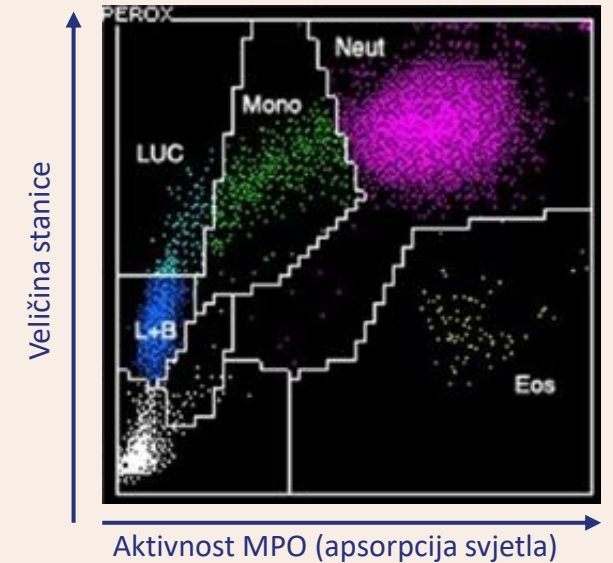
## Baso kanal

Liziranje citoplazme svih leukocita, osim bazofila. Detektira se razlika u obliku i kompleksnosti jezgre (MN, PMN)

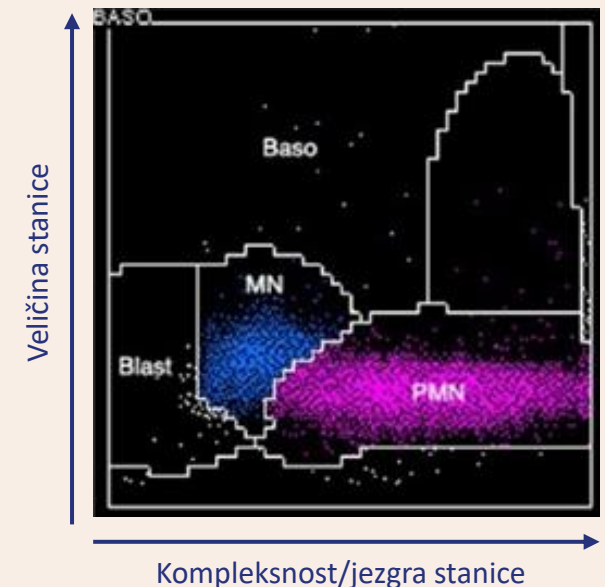
## RBC/PLT kanal

Reagens sadrži natrij dodecil sulfat i glutaraldehid kojima se Erc i Trc izovolumetrijski zaokružuju.

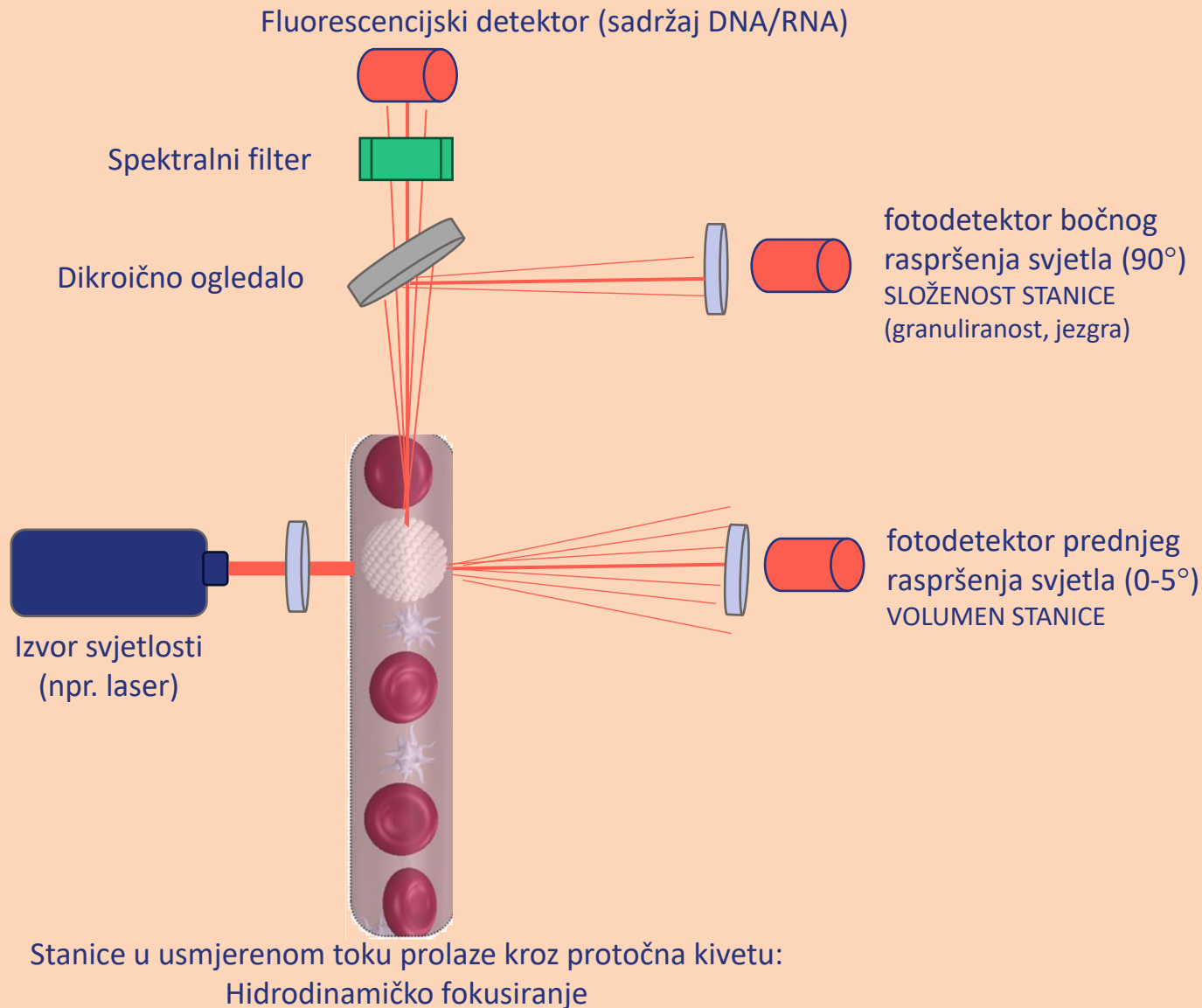
Perox kanal - dijagram raspršenja



Baso kanal - dijagram raspršenja



# Optička metoda + fluorescencija



Sysmex XN / XR serija

Mindray BC 6000/6200/6800

Integracija detektora fluorescentnog signala u sklopu optičkog sustava.

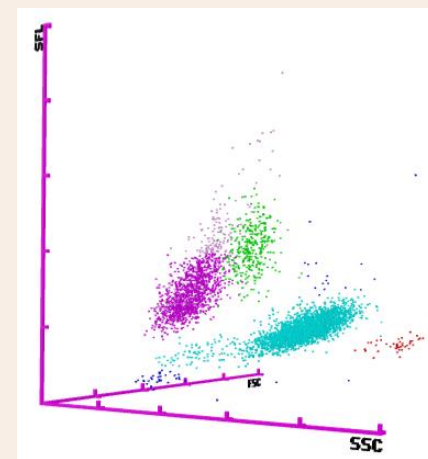
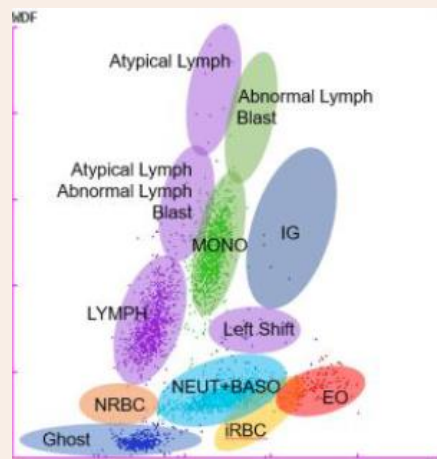
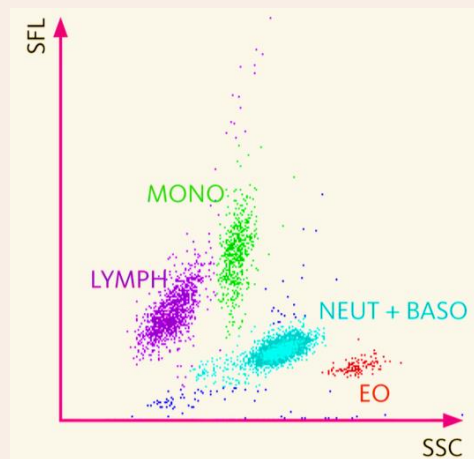
Stanice se boje fluorescentnom bojom, a intenzitet obojenja ovisi o sadržaju DNA/RNA.

Populacija nezrelih granulocita (engl. Immature granulocyte – IG) kao šesta subpopulacija leukocita.

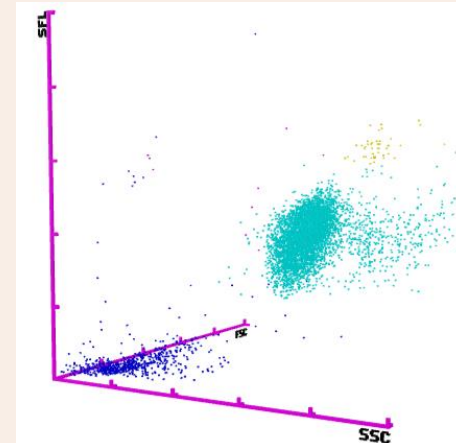
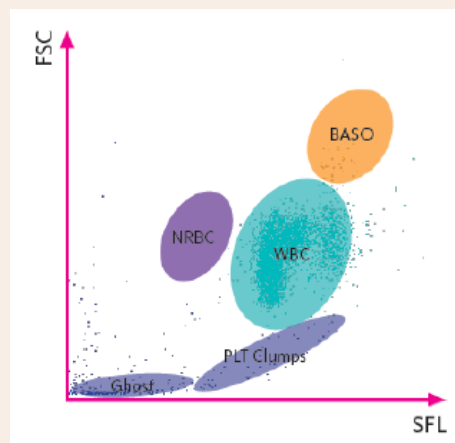
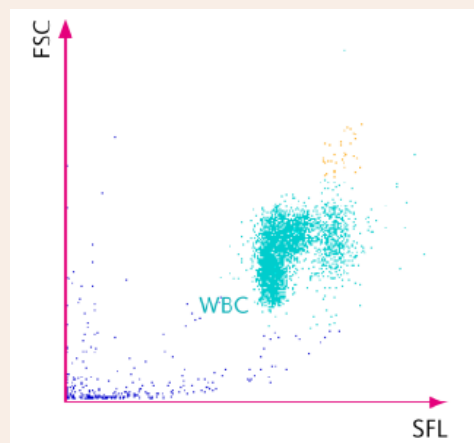


# Optička metoda + fluorescencija

Diff kanal – 2D i 3D dijagrami raspršenja



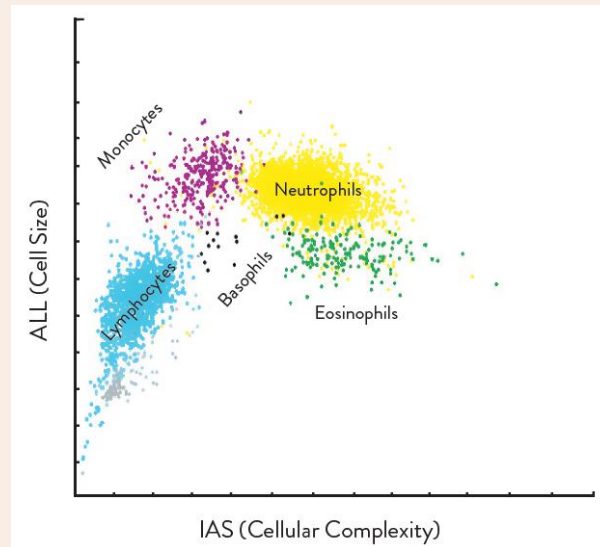
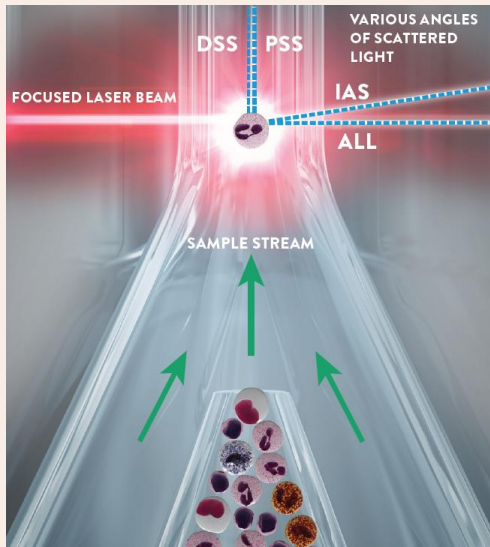
Baso kanal – 2D i 3D dijagrami raspršenja



# Optička metoda

**Abbott MAPSS™ (Multi-Angle Polarized Scatter Separation) tehnologija**

Diferenciranje leukocita se odvija u jednom kanalu.



**Abbott Alinity hq**

**MAPSS™ tehnologija**

Optical detectors used by Alinity hq as part of the advanced MAPSS™ technology

Detector	Angle	Function	Application
All	0-2.5°	Cell size	All modes
IAS	2.5 – 7.5°	Cell content/ complexity	All modes
IAS1	2.5 – 4.5°	Cellular HGB content	RBC, PLT and RETIC
IAS2	4.5 – 5.5°	Cell volume	RBC, PLT and RETIC
IAS3	5.5 – 7.5°	Granularity (PLT)	RBC, PLT and RETIC
PSS	90° (pol)	Internal complexity (Nuclear segmentation and cytoplasmic granulation)	All modes
DSS	90° (depol)	Eosinophil granules	WBC differential (Eosinophils)
FL1	90° (fluor)	Fluorescence (dye uptake)	CBC (nucleated cells); RETIC

0° or Axial Light Loss (ALL): veličina stanice

0° to 10° Intermediate Angle Scatter (IAS): složenost stanice

90° Polarized Side Scatter (PSS): kompleksnost jezgre

90° Depolarized Side Scatter (DSS): eozinofilne granule

# Poruke upozorenja

Softveri hematoloških analizatora koriste kompleksne algoritme kako bi definirali optimalnu poziciju diskriminatora populacije svih stanica.

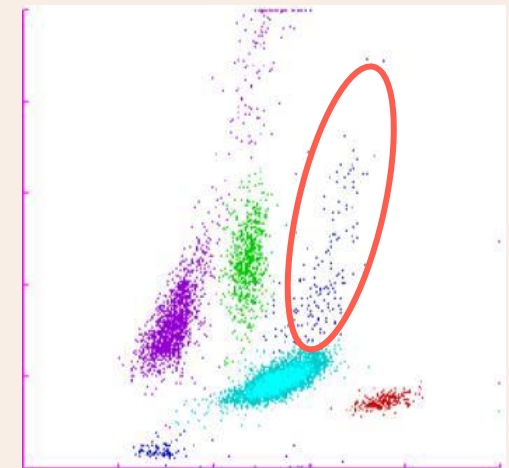
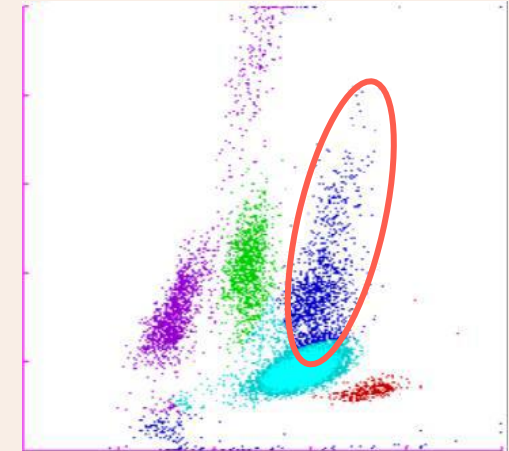
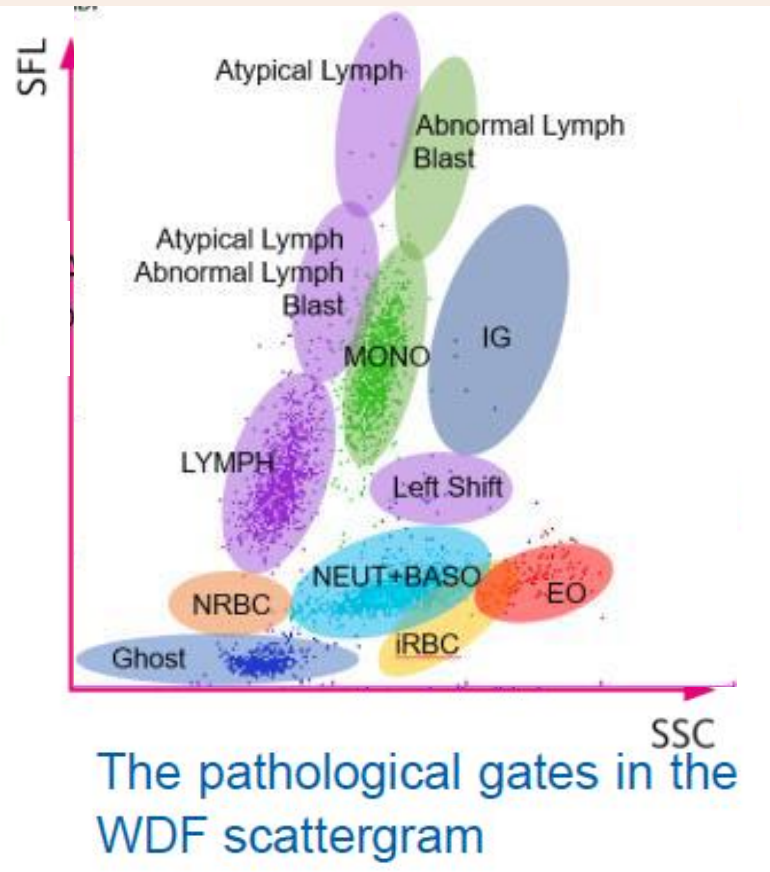
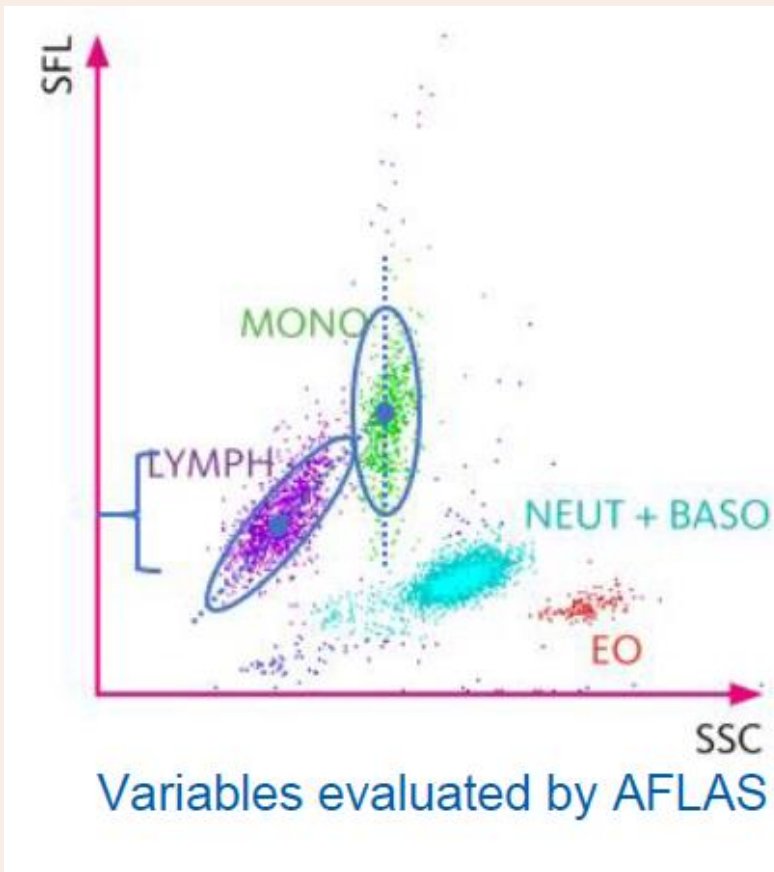
Kada se pojavi populacija stanica koja se ne može raspodijeliti niti u jednu od predefiniranih kategorija, pojavljuju se poruke upozorenja.

Poruke upozorenja mogu biti povezane uz brojčanu vrijednost (iznad ili ispod referentnog raspona), uz kvalitativnu vrijednost (abnormalna pozicija populacije stanica u dijagramu raspršenja ili histogramu) ili uz sistemski problem u radu analizatora.

Flag Message	Indication	Criteria
WBC scattergram abnormal	WBC scattergram abnormal	The distribution of DIFF channel scattergram is abnormal
RBC Agglutination?	RBC results possibly inaccurate	Calculate and compare special parameters
Turbidity/HGB Interference?	Abnormal HGB or there may be interference	Calculation and comparison of special parameters.
Iron Deficiency?	May indicate iron deficiency anemia	Calculation and comparison of special parameters
Fragments?	Possible presence of RBC fragments	Presence of abnormally distributed dots in sensitive region of the RET channel
PLT Scattergram Abn.	Abnormal distribution of PLT scattergram	The distribution of PLT scattergram is abnormal
PLT Histogram Abn.	Abnormal distribution of PLT histogram	The distribution of PLT histogram is abnormal
Thrombopenia	PLT low	$PLT < 60 \times 10^9/L$
Thrombocytosis	PLT high	$PLT > 600 \times 10^9/L$
PLT Clump?	There may be PLT clump	Calculation and comparison of special parameters
RBC Histogram Abn.	Abnormal distribution of RBC histogram	Abnormal distribution of RBC histogram

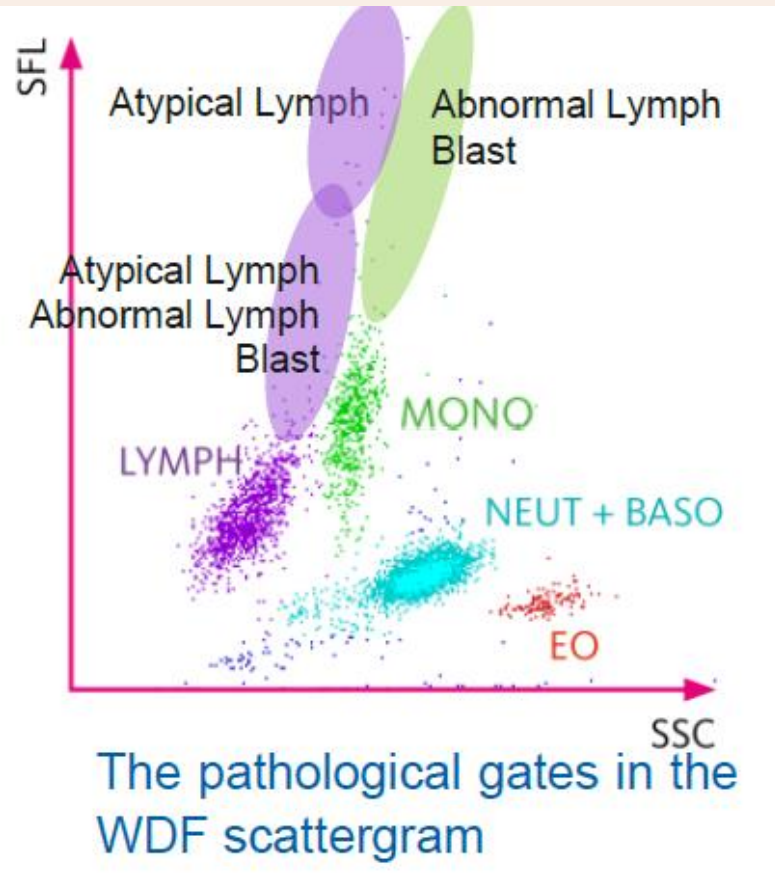
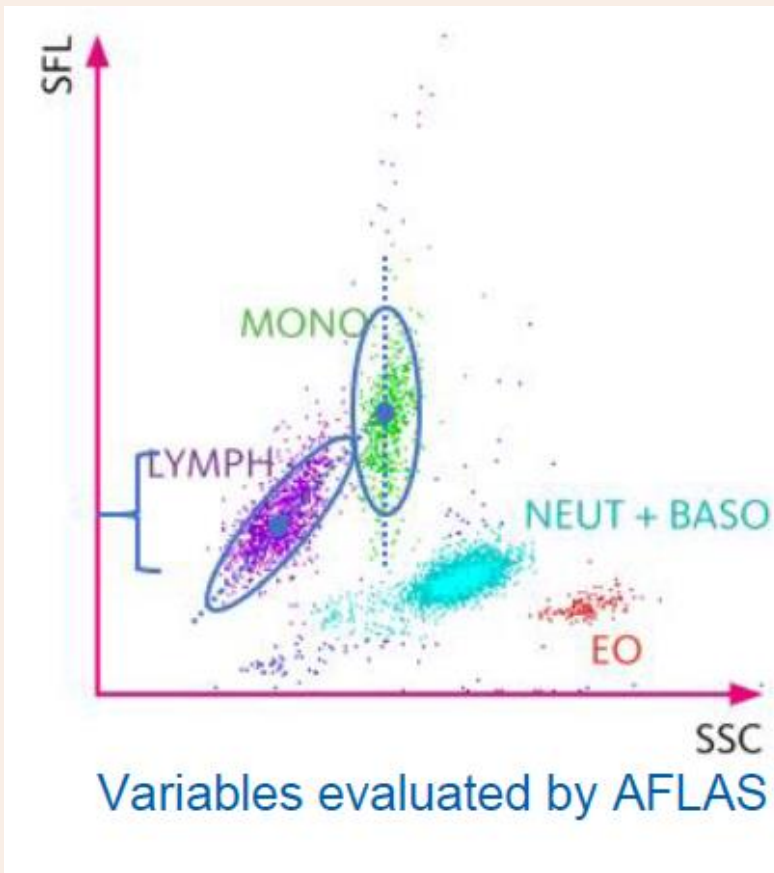
# Poruke upozorenja - primjeri

IG Present

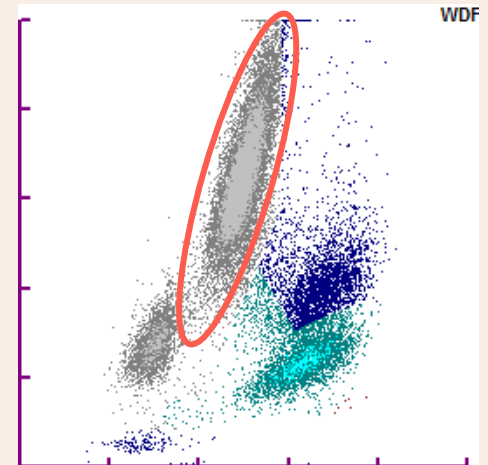
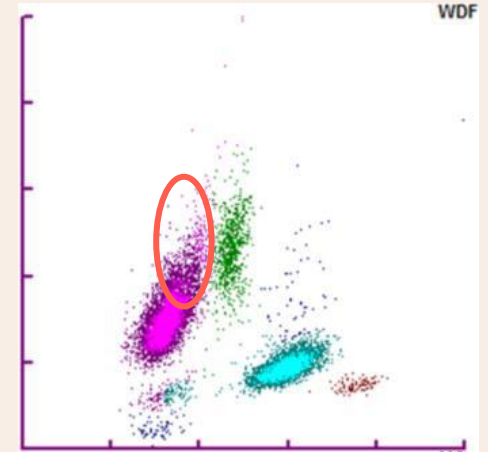




# Poruke upozorenja - primjeri



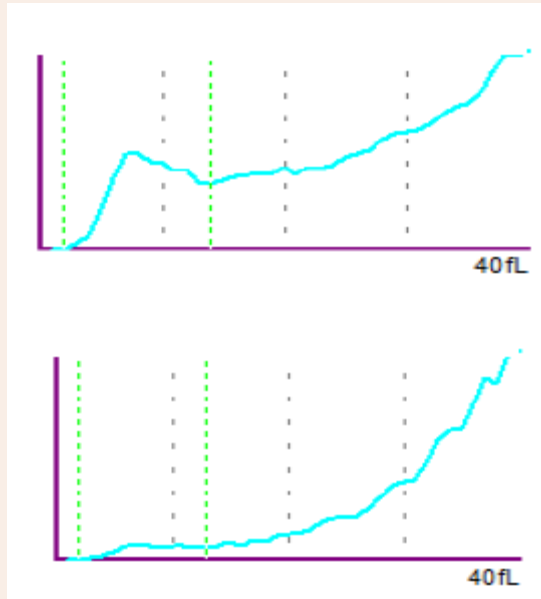
Blasts/Abn Lympho?



# Poruke upozorenja - primjeri

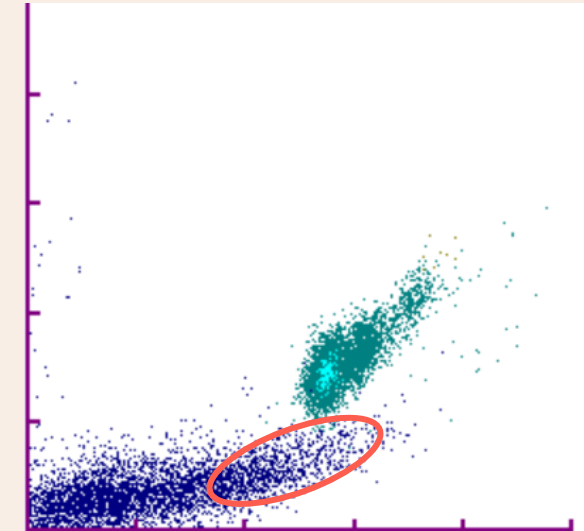
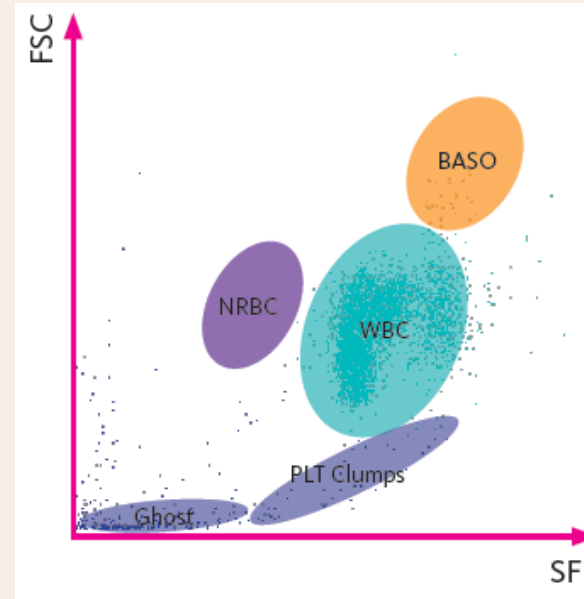
## Plt Abn Distribution

PLT Histogram



## Plt Clumps

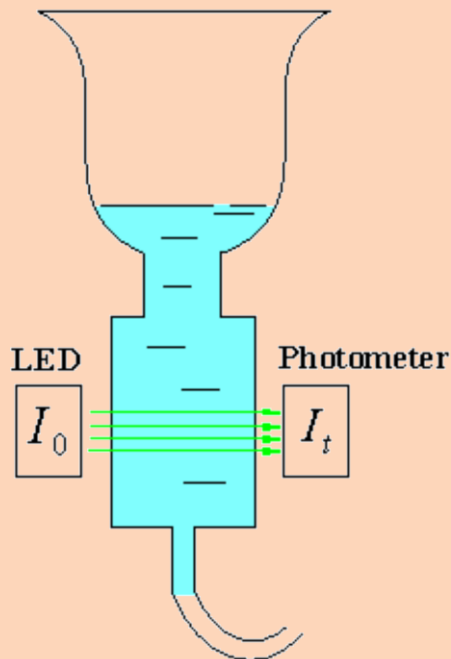
WNR scattergram (SFL-SSC)



# Metoda određivanja hemoglobina

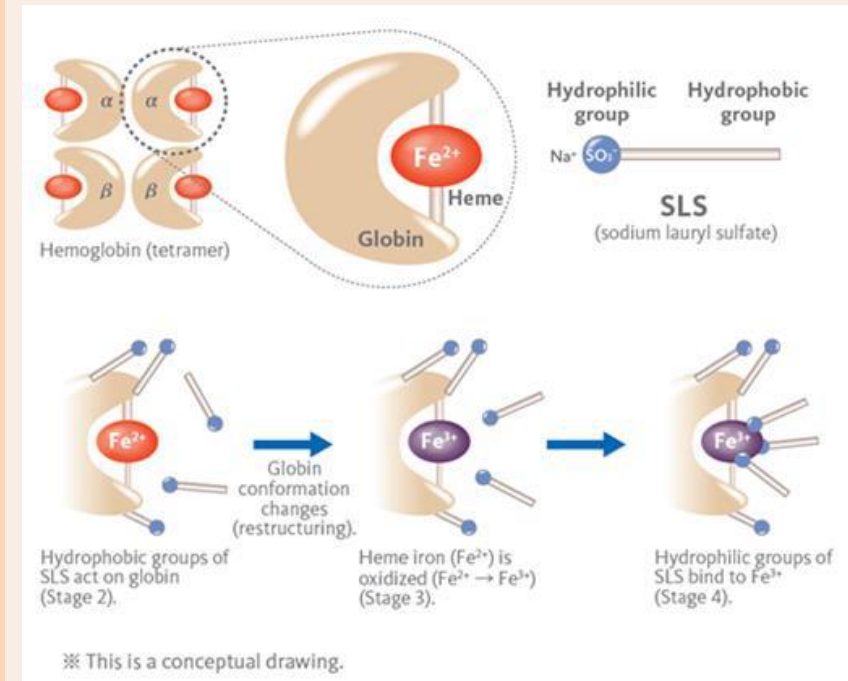
## Referentna metoda: ICSH metoda s hemoglobincijanidom

Drabkinov reagens (K-fericijanid, K-cijanid, pH 7,0-7,4). Sve forme hemoglobina, uključujući methemoglobin i karboksihemoglobin pretvaraju u stabilni oblik hemoglobincijanida. Intezitet nastalog obojenja mjeri se spektrofotometrijski na 540 nm.



## Hematološki analizatori - modifikacija referentne metode:

- Neionski detergents – liziranje stanica i smanjenje zamućenja
- Promjena pH
- Promjena koncentracije reagensa
- Zamjena cijanida s drugim reagensima





## KAPACITET ANALIZATORA

- 20 / 30/ 40 / 60 / 80 /100 /120 KKS/h
- Kapacitet se smanjuje ako se KKS kombinira s RTC
- Kapacitet se povećava primjenom integriranih rješenja

## MODULI ZA UZORKOVANJE



## VOLUMEN UZORKA

- Definirani minimalni volumen uzorka (otvorena/zatvorena epruveta)
- Definirani mrtvi volumen uzorka
- Mogućnost rada s mikrouzorcima

## INTEGRIRANA RJEŠENJA



## MJERNI RASPON LINEARNOST

Parameter	Units	Analytical Measuring Range
WBC	$\times 10^3$ cells/ $\mu$ L	0.050–2.000 >2.000–100.000 >100.000–400.000
RBC	$\times 10^6$ cells/ $\mu$ L	0.005–8.500
HGB	g/dL	0.10–25.50
MCV	fL	50.00–150.00
RDW	%	10.00–40.00
RDW-SD	fL	15.00–150.00
PLT	$\times 10^3$ cells/ $\mu$ L	3.0–3000.0
MPV	fL	5.00–25.00

## PRECIZNOST

WBC	$\geq 4 \times 10^9/L$	$\leq 2.5\%$
RBC	$\geq 3.5 \times 10^{12}/L$	$\leq 1.5\%$
HGB	(110-180)g/L	$\leq 1.0\%$
MCV	(80-100)fL	$\leq 1.0\%$
HCT	(30~50)%	$\leq 1.5\%$
MCH	—	$\leq 1.5\%$
MCHC	—	$\leq 1.5\%$
RDW-SD	—	$\leq 2.0\%$
RDW-CV	—	$\leq 2.0\%$
PLT	$\geq 100 \times 10^9/L$	$\leq 4.0\%$

## DOSTUPNI BROJ TESTOVA

**Standardni panel:** Eritrociti, Hemoglobin, Hematokrit, MCV, MCH, MCHC, RDW, Trombociti, MPV, Leukociti, Neutrofili, Limfociti, Monociti, Eozinofili, Bazofili

**Dodatni testovi:** Retikulociti, frakcija nezrelih retikulocita (IRF), RDW-SD, eritroblasti, PDW, frakcija nezrelih trombocita (IPF), nezreli granulociti (IG)

**RUO (Research use only) testovi:** %mikro/makro, %hipo/hiper, %/# fragmentirani eritrociti, sadržaj hemoglobina u retikulocitima, NLR, PLR...

**Analiza tjelesnih tekućina:** Leukociti, ukupni broj stanica s jezgrom (TNC), %MN, %PMN, Eritrociti

**Testovi jedinstveni za analizator:** MDW

## KONTROLE & KALIBRATORI

- Svi hematološki analizatori nude kontrolne uzorke u više razina
- Dostupnost kalibratora ovisi o tipu analizatora

# Zaključne poruke za kraj

- Upoznajte svoj hematološki analizator, sve mogućnosti i ograničenja, iskoristite njegov puni kapacitet
- Osigurajte kontinuiranu edukaciju osoblja
- Definirajte jasne laboratorijske algoritme

**...i voljet ćete hematologiju još i više...**



**Hvala na  
pozornosti!**