Blutbild 2012: Automatisiert oder manuell?

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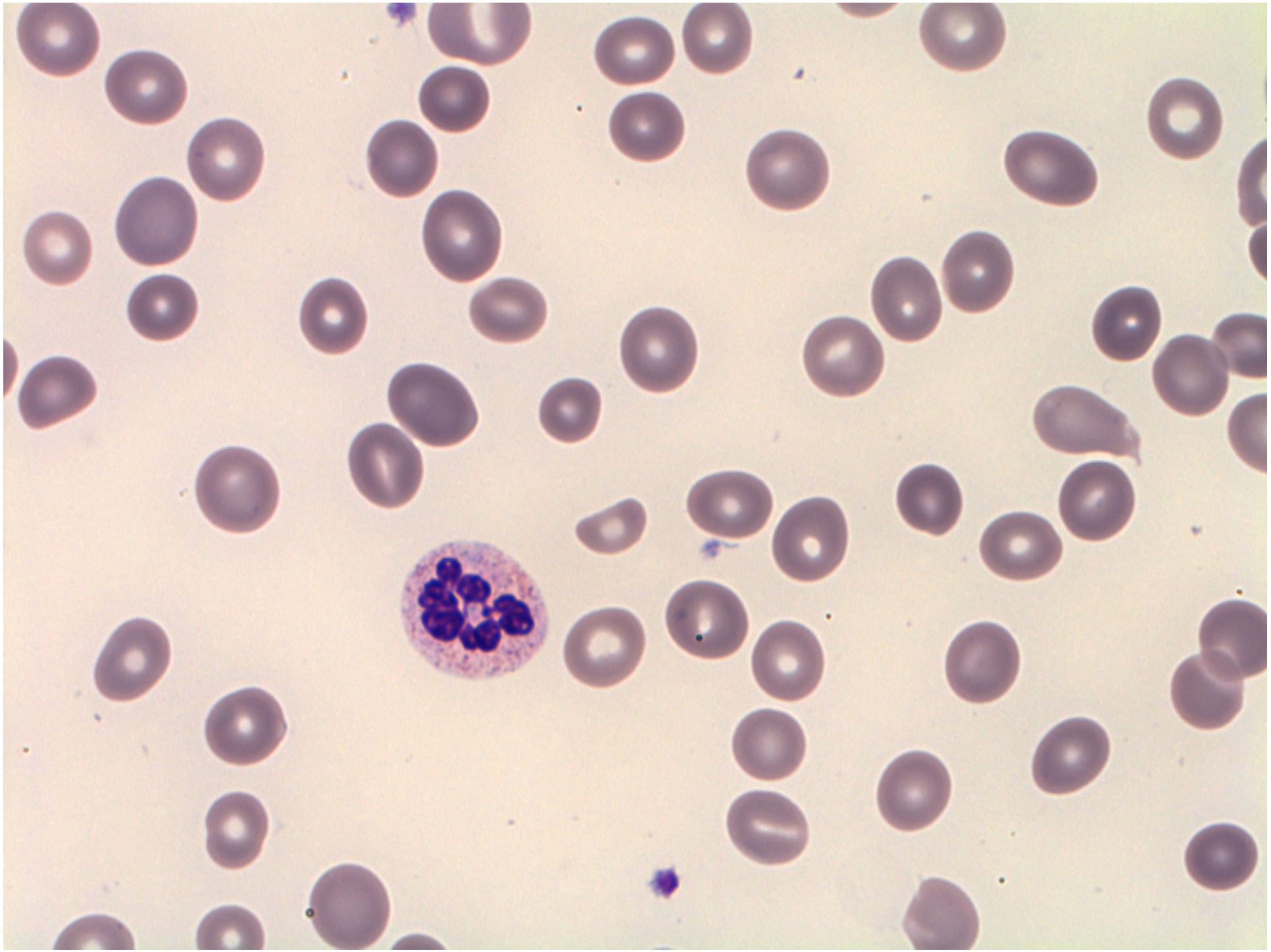
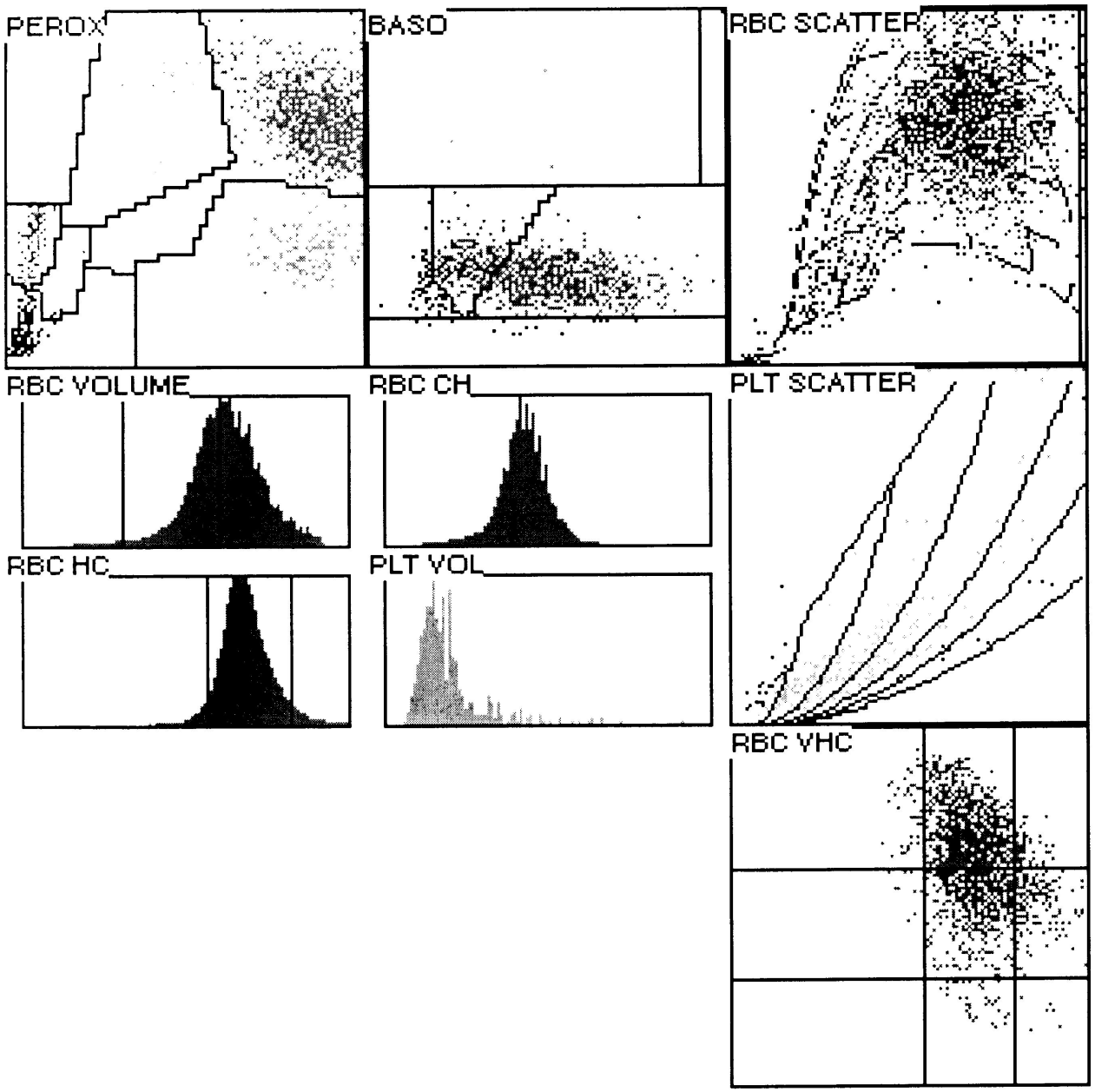
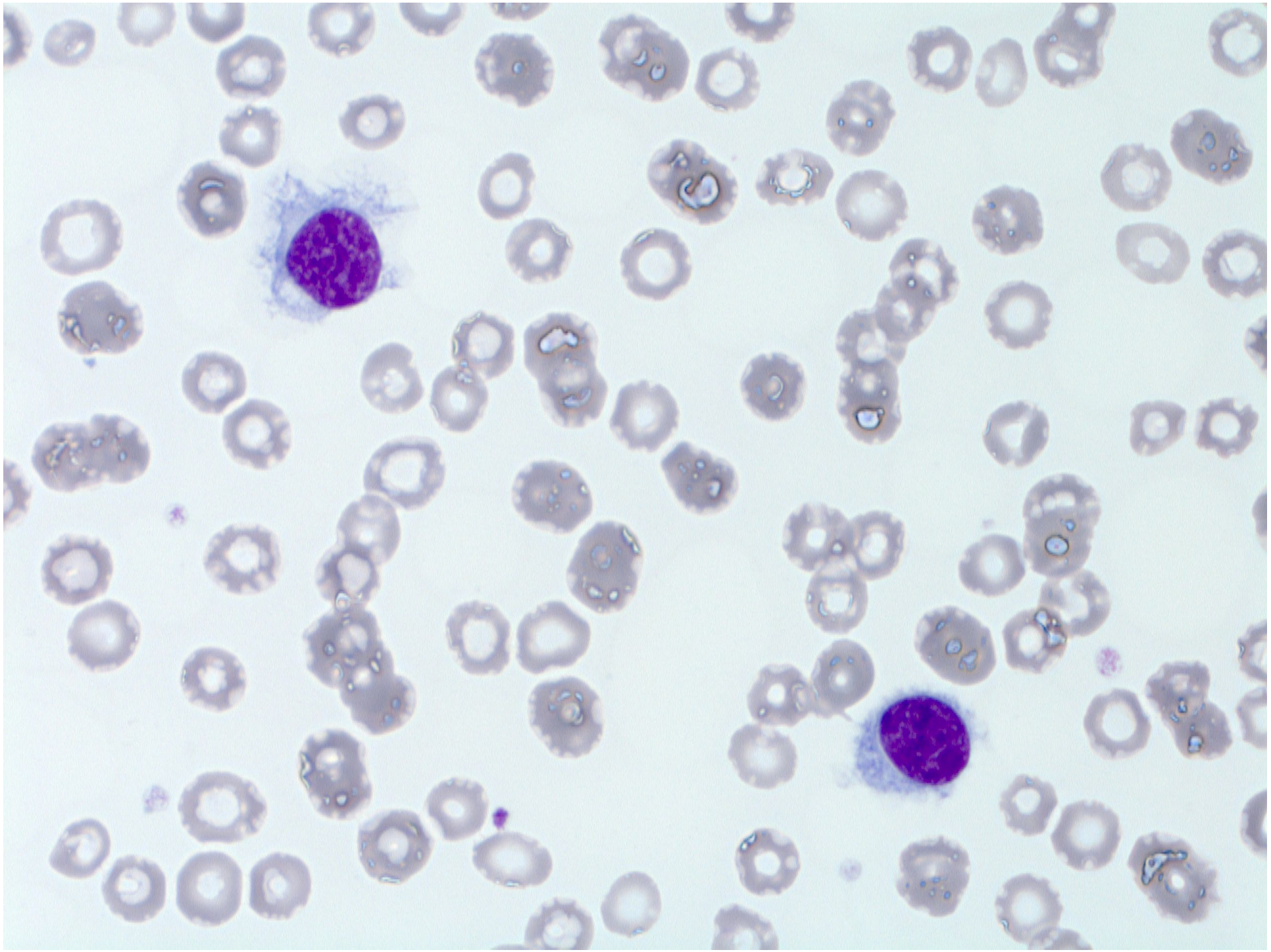
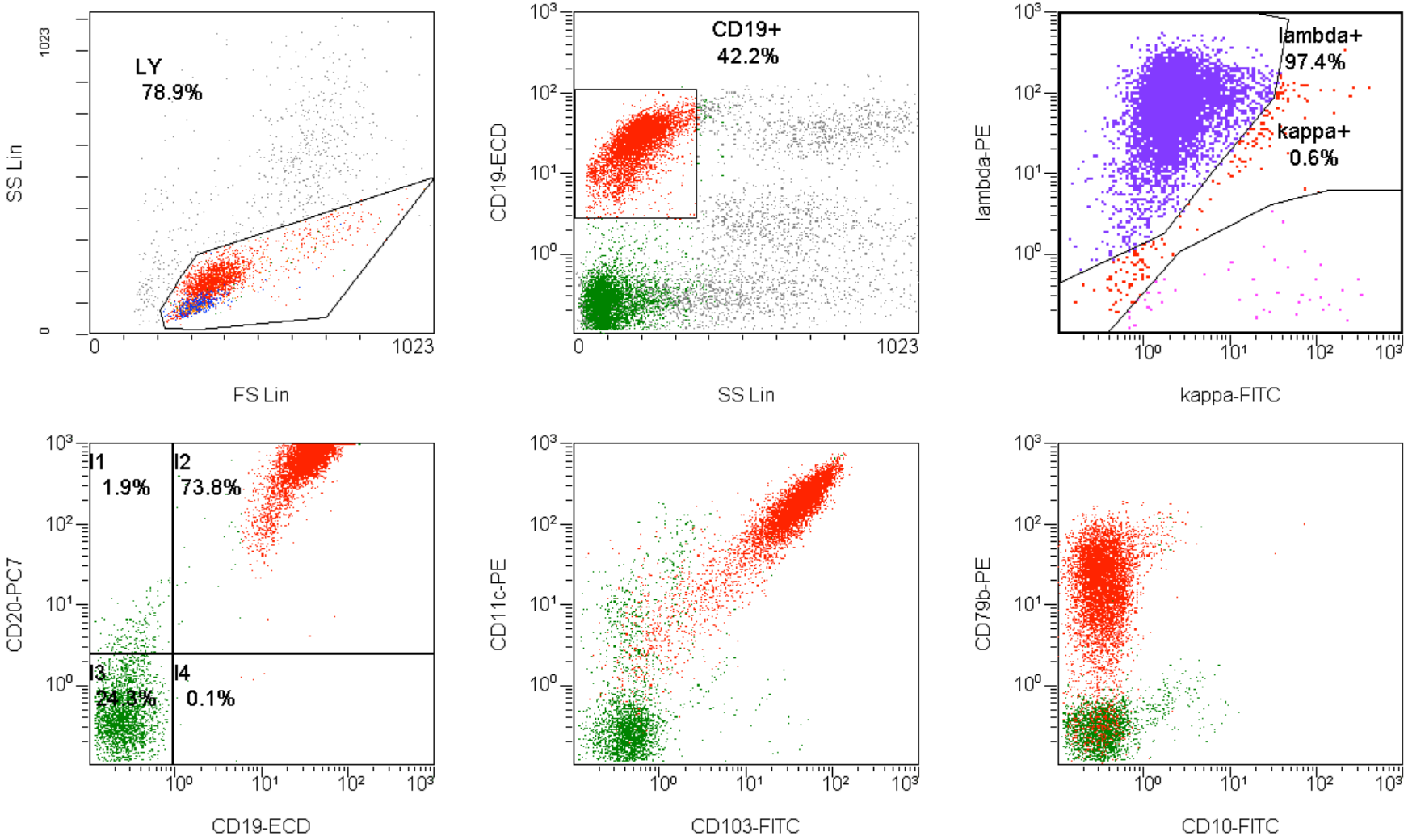
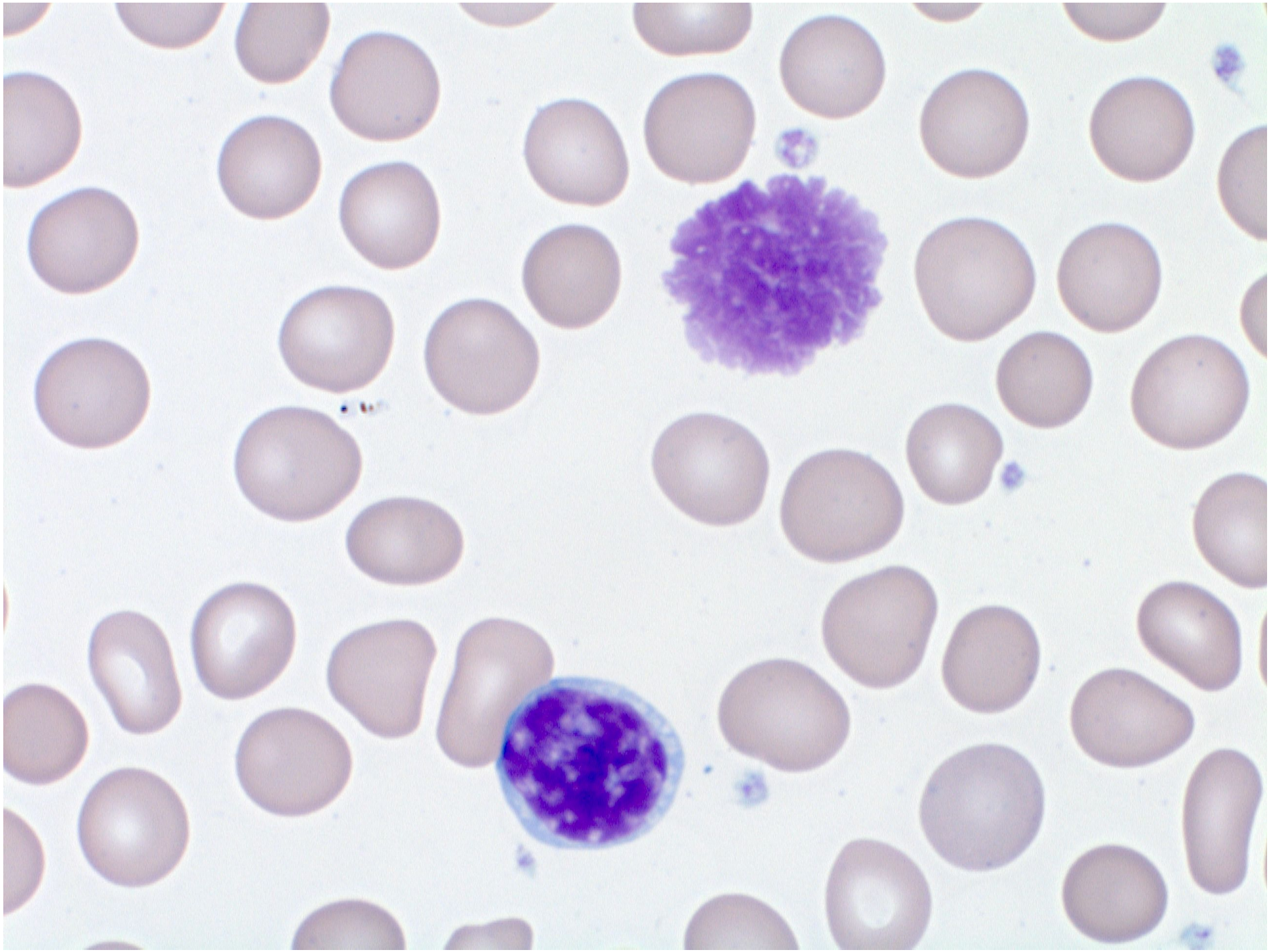
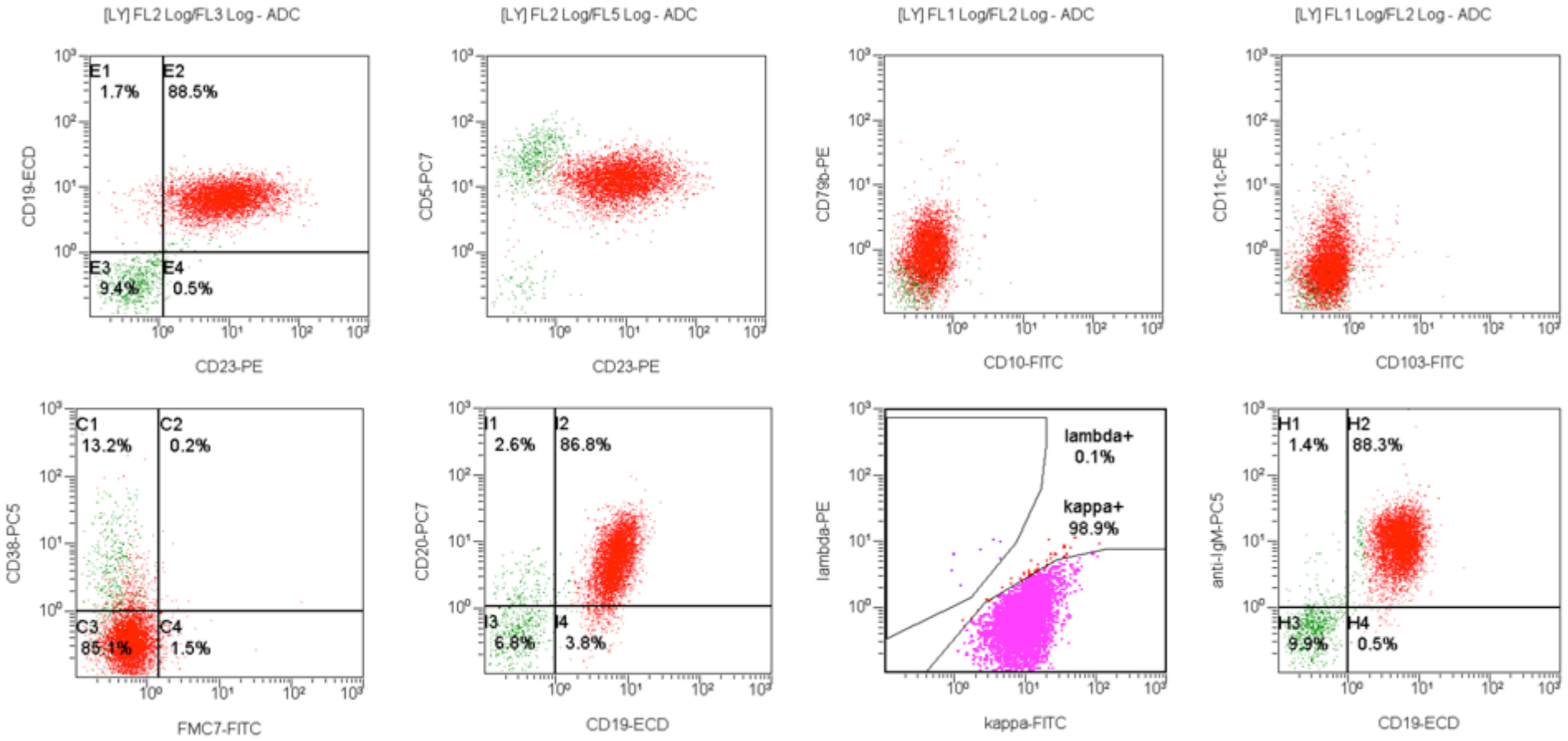


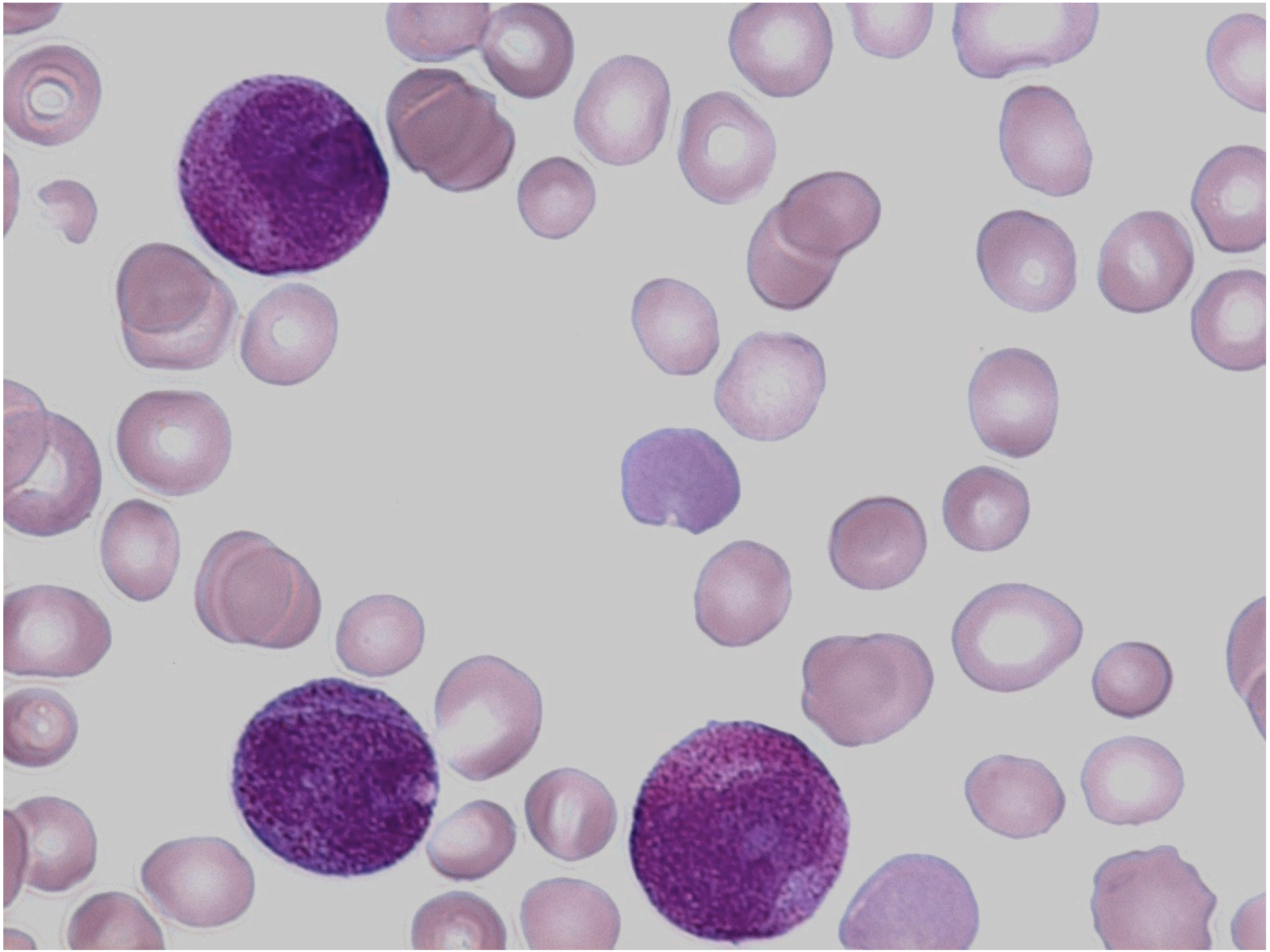

PEROX
BASO
RBC SCATTER
RBC VOLUME
RBC CH
PLT SCATTER
RBC HC
PLT VOL
RBC VHC

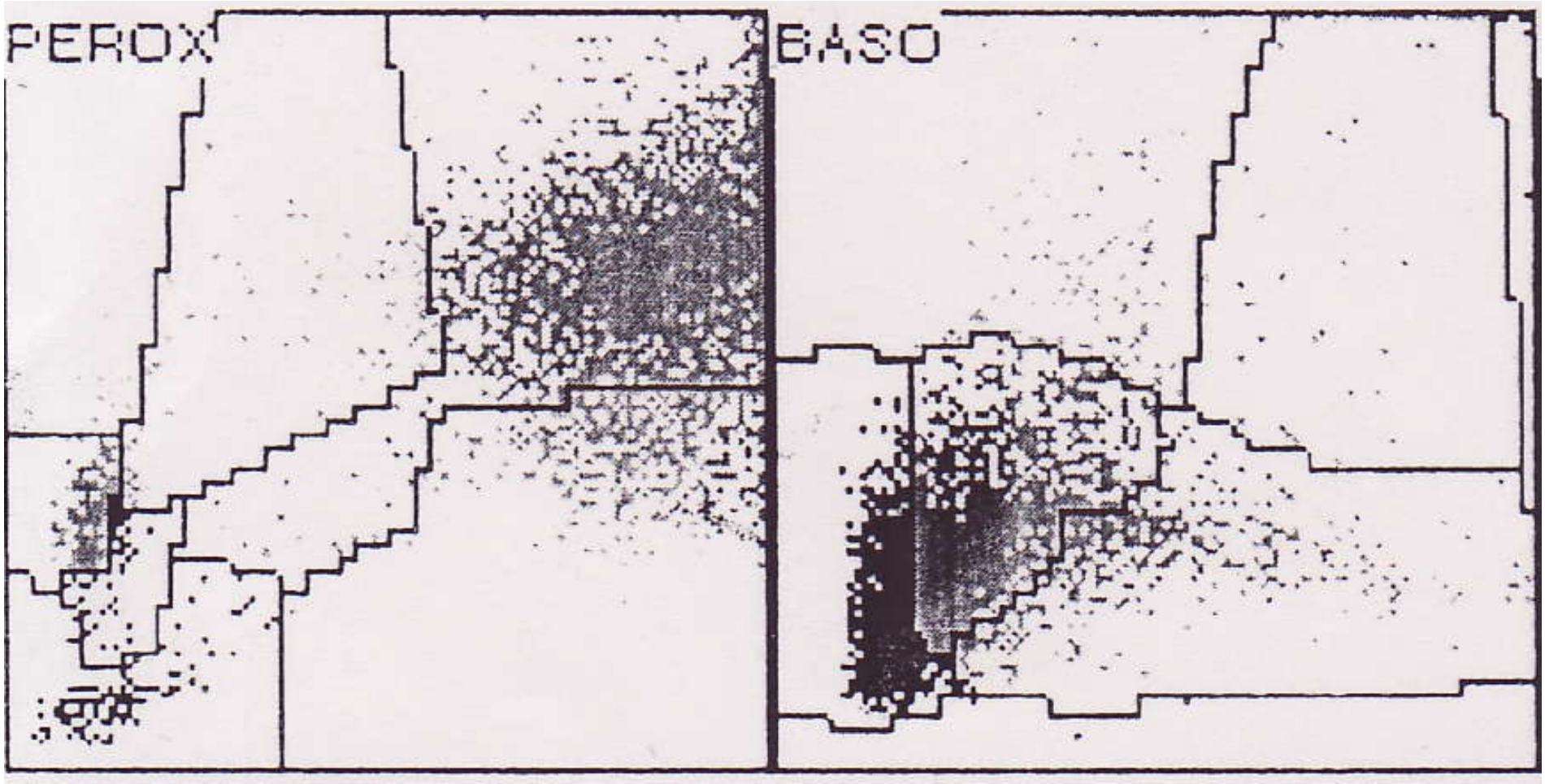



LY
78.9%
SS Lin
FS Lin
0
1023
CD19+
42.2%
CD19-ECD
SS Lin
lambda+
97.4%
kappa+
0.6%
lambda-PE
kappa-FITC
I1
1.9%
I2
73.8%
I3
24.3%
I4
0.1%
CD20-PC7
CD19-ECD
CD11c-PE
CD103-FITC
CD79b-PE
CD10-FITC




[LY] FL2 Log/FL3 Log - ADC
E1
1.7%
E2
88.5%
E3
9.4%
E4
0.5%
CD19-ECD
CD23-PE
[LY] FL2 Log/FL5 Log - ADC
CD5-PC7
CD23-PE
[LY] FL1 Log/FL2 Log - ADC
CD79b-PE
CD10-FITC
[LY] FL1 Log/FL2 Log - ADC
CD11c-PE
CD103-FITC
C1
13.2%
C2
0.2%
C3
85.1%
C4
1.5%
CD38-PC5
FMC7-FITC
I1
2.6%
I2
86.8%
I3
6.8%
I4
3.8%
CD20-PC7
CD19-ECD
lambda+
0.1%
kappa+
98.9%
lambda-PE
kappa-FITC
H1
1.4%
H2
88.3%
H3
9.9%
H4
0.5%
anti-IgM-PC5
CD19-ECD




PEROX
BASO


1
2
3
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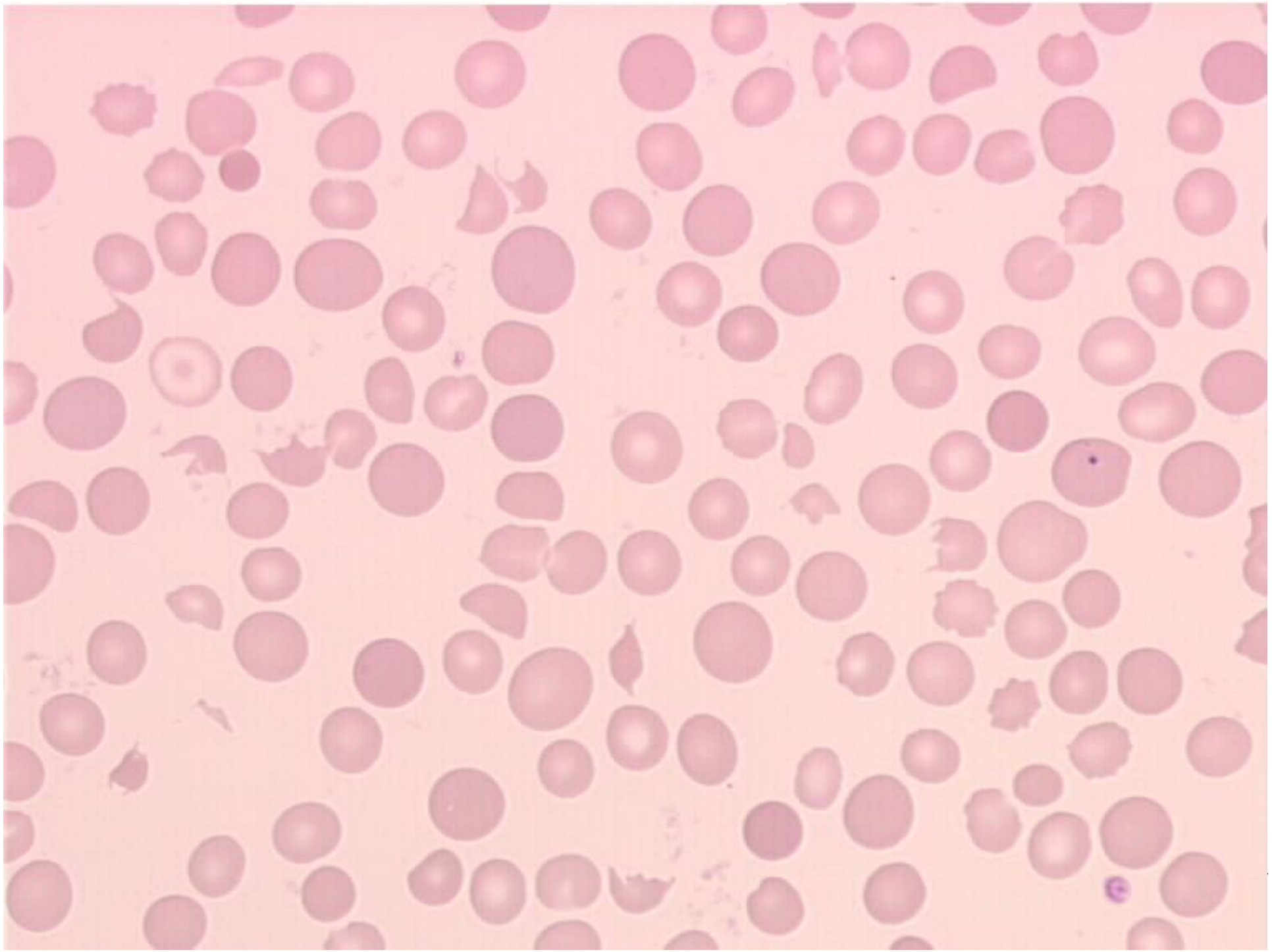
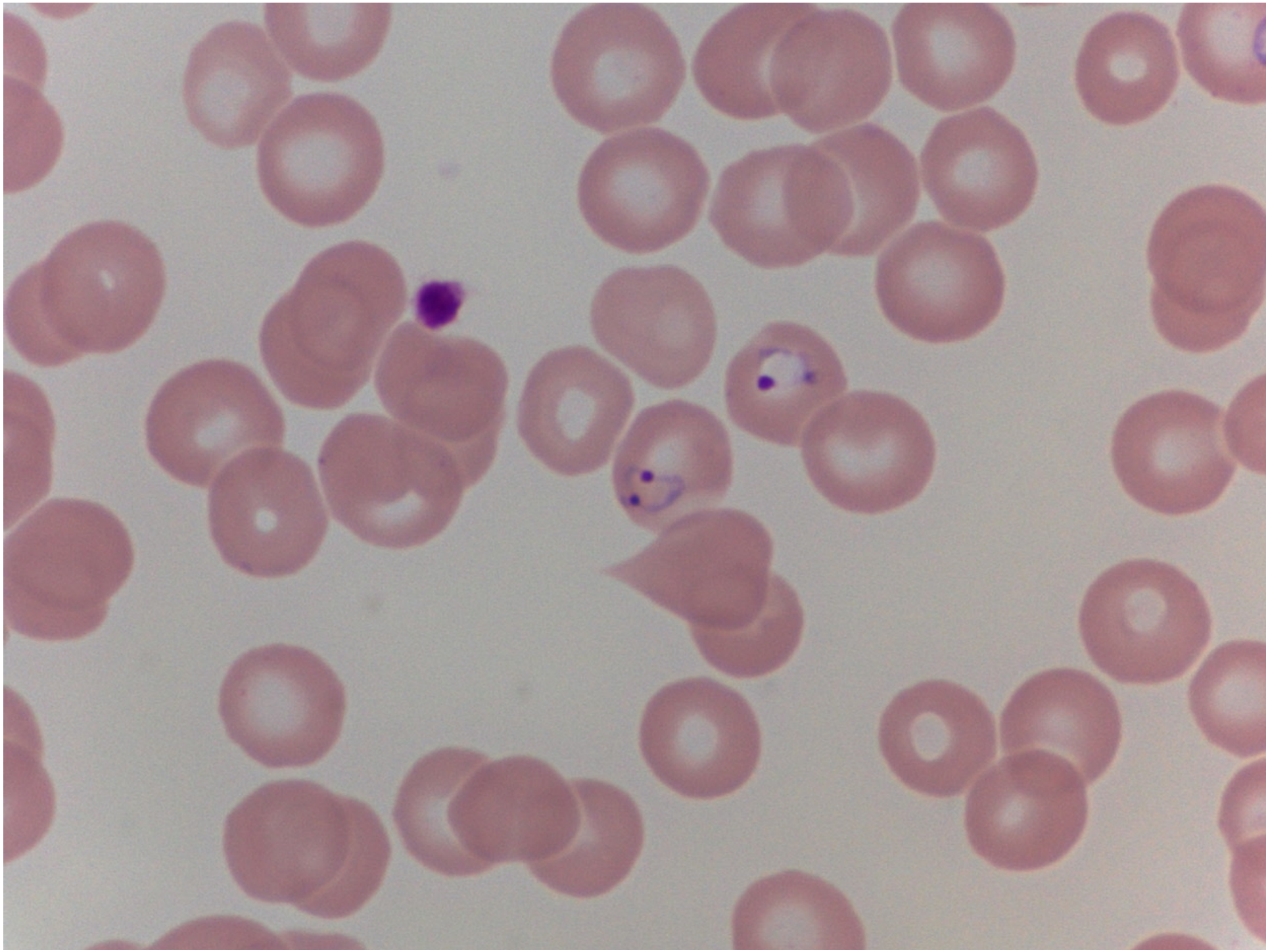
22
Y
X

IS THERE STILL A ROLE FOR THE MANUAL BLOOD COUNT?





Indication for examination of a blood smear

Table 1. Clinical Indications for Examination of a Blood Smear.

Features suggestive of anemia, unexplained jaundice, or both
Features suggestive of sickle cell disease — dactylitis or sudden splenic enlargement and pallor in a young child or, in an older child or adult, limb, abdominal, or chest pain
Features suggestive of thrombocytopenia (e.g., petechiae or abnormal bruising) or neutropenia (e.g., unexpected or severe infection)
Features suggestive of a lymphoma or other lymphoproliferative disorder — lymphadenopathy, splenomegaly, enlargement of the thymus (a mediastinal mass on radiology) or other lymphoid organs, skin lesions suggestive of infiltration, bone pain, and systemic symptoms such as fever, sweating, itching, and weight loss
Features suggestive of a myeloproliferative disease — splenomegaly, plethora, itching, or weight loss
Suspicion of disseminated intravascular coagulation*
Acute or recent-onset renal failure or unexplained renal enlargement, particularly in a child
On retinal examination, hemorrhages, exudates, signs of hyperviscosity, or optic atrophy
Suspicion of a bacterial or parasitic disease that can be diagnosed from a blood smear
Features suggestive of disseminated nonhematopoietic cancer — weight loss, malaise, bone pain
General ill health, often with malaise and fever, suggesting infectious mononucleosis or other viral infection or inflammatory or malignant disease

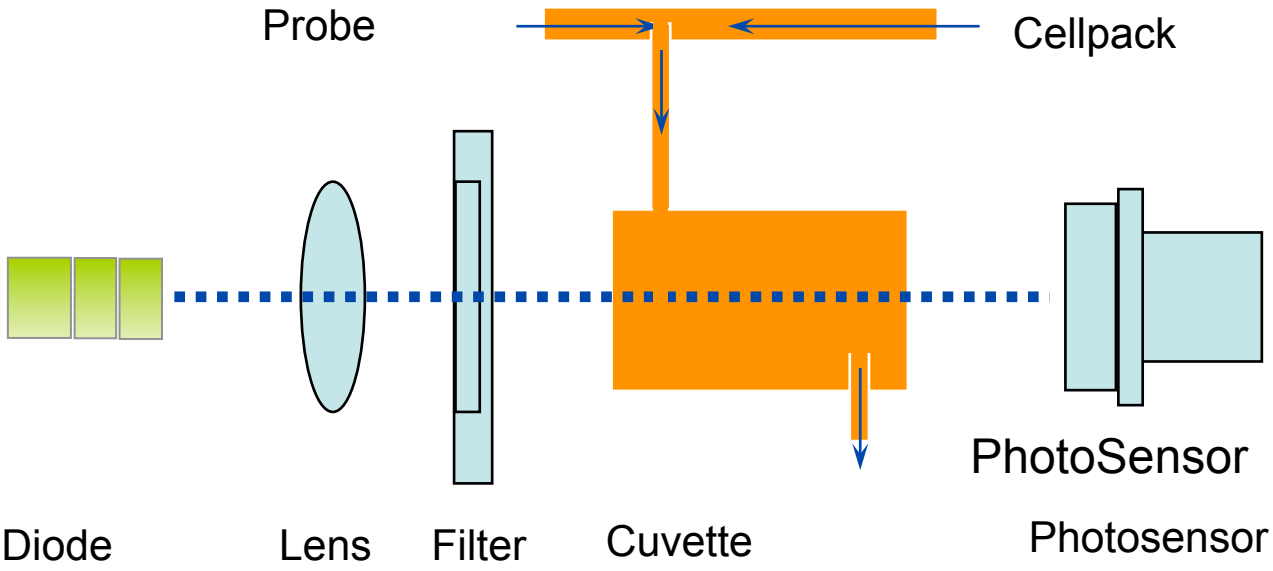
Bain BJ. Diagnosis from the blood smear. *N Engl J Med*. 2005;353(5):498–507.

The blood counters

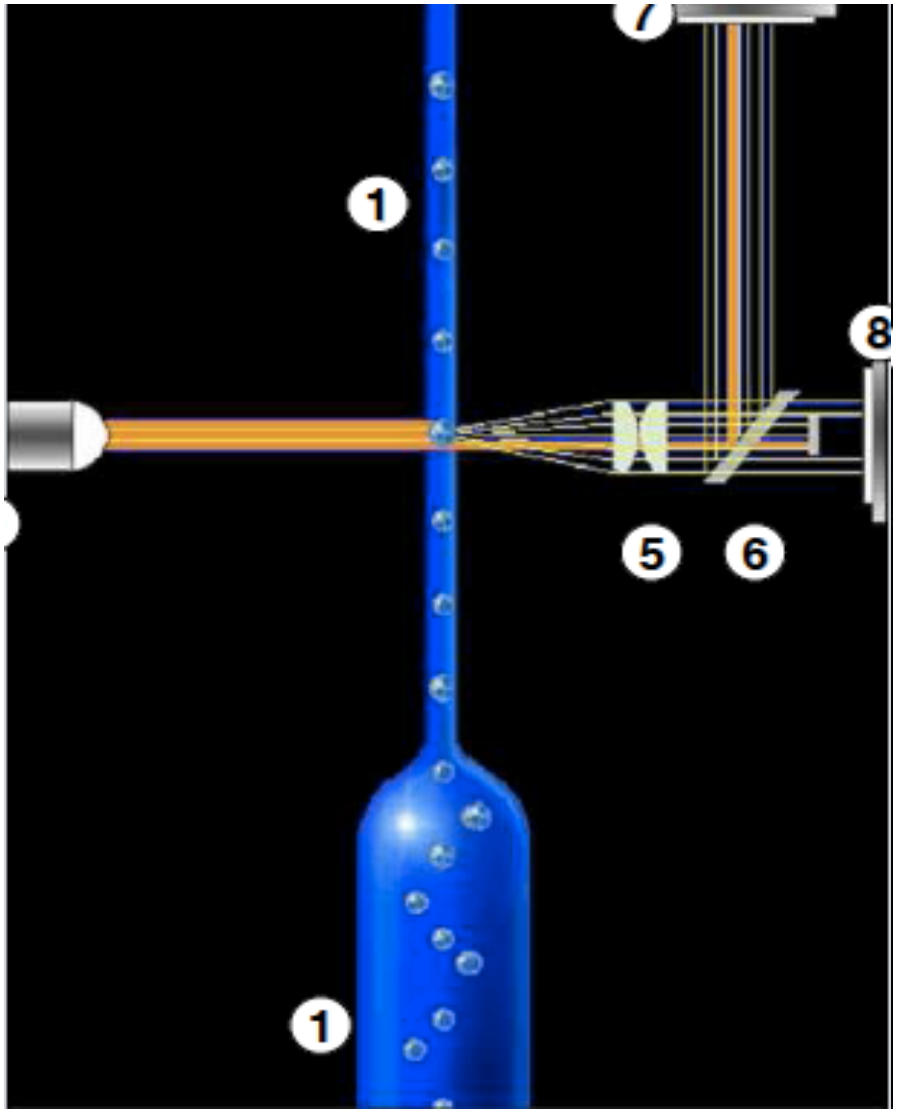
Principles of cell counters

- Light absorption
- Light scatter
- Impedance
- Fluorescence
- Electrical conductivity

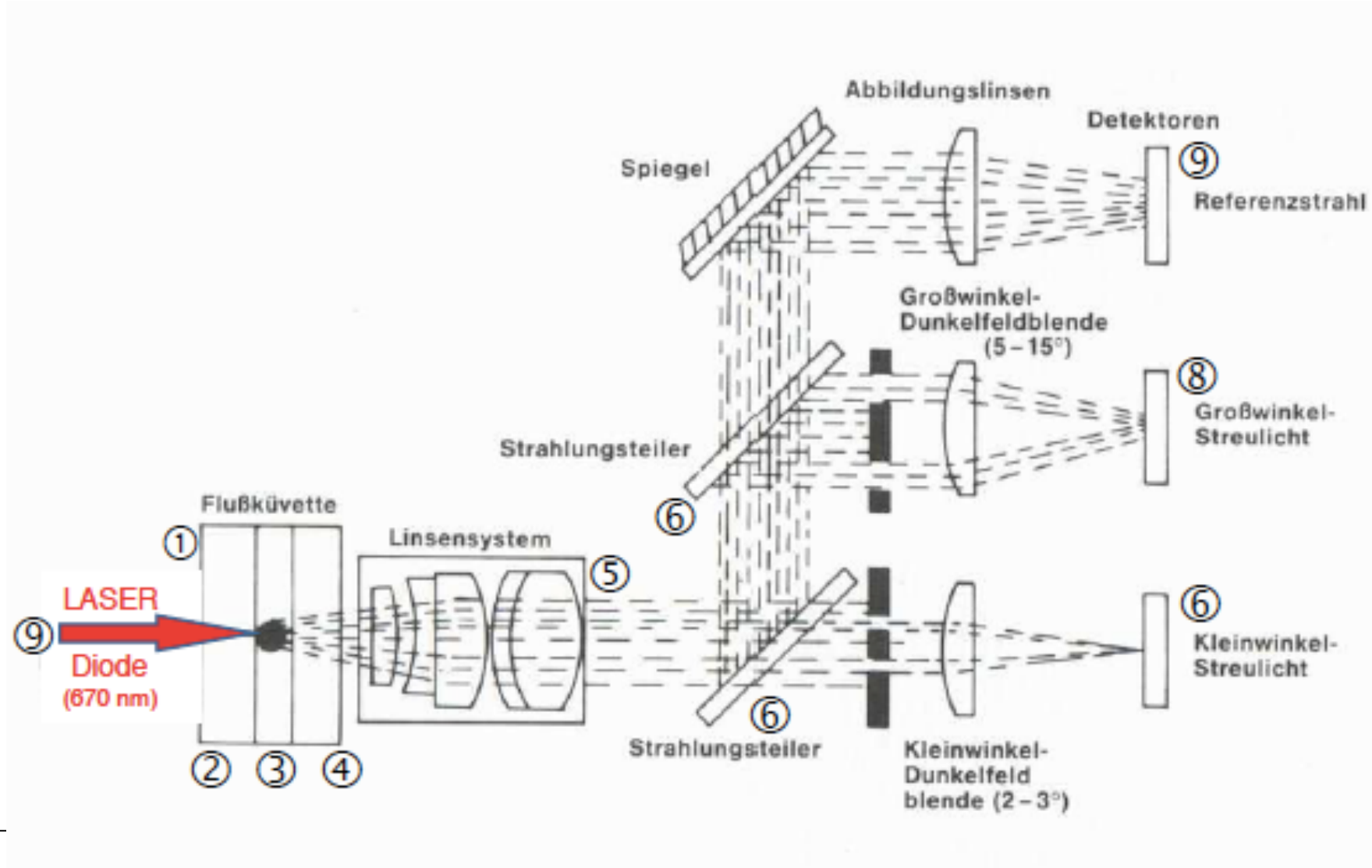
Hemoglobin photometry



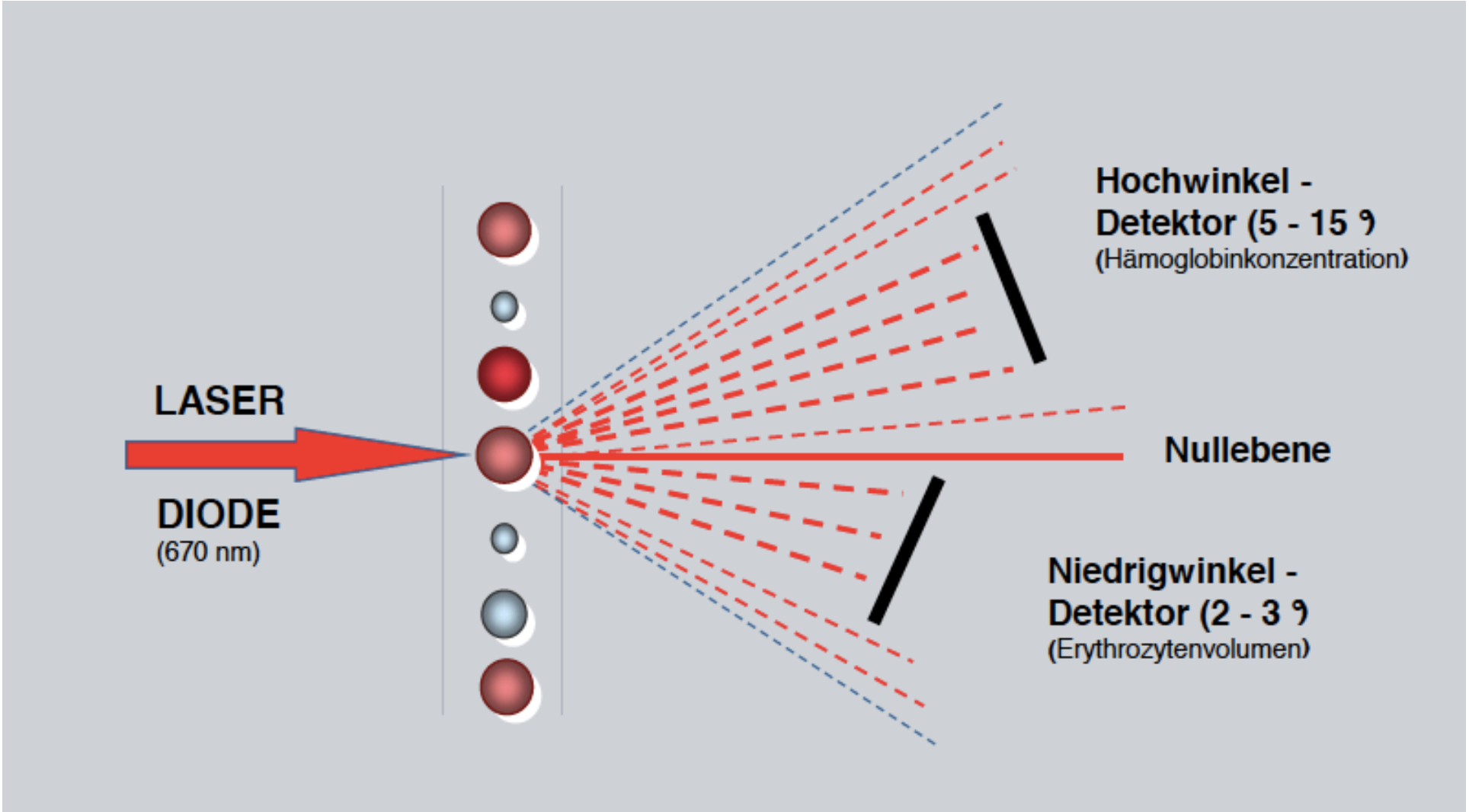
WBC Count: Light scattering



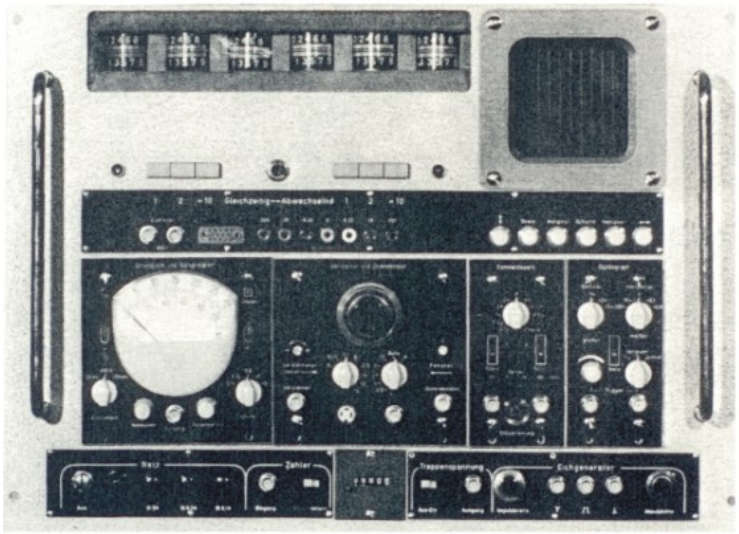
ADVIA: RBC/PLT channel



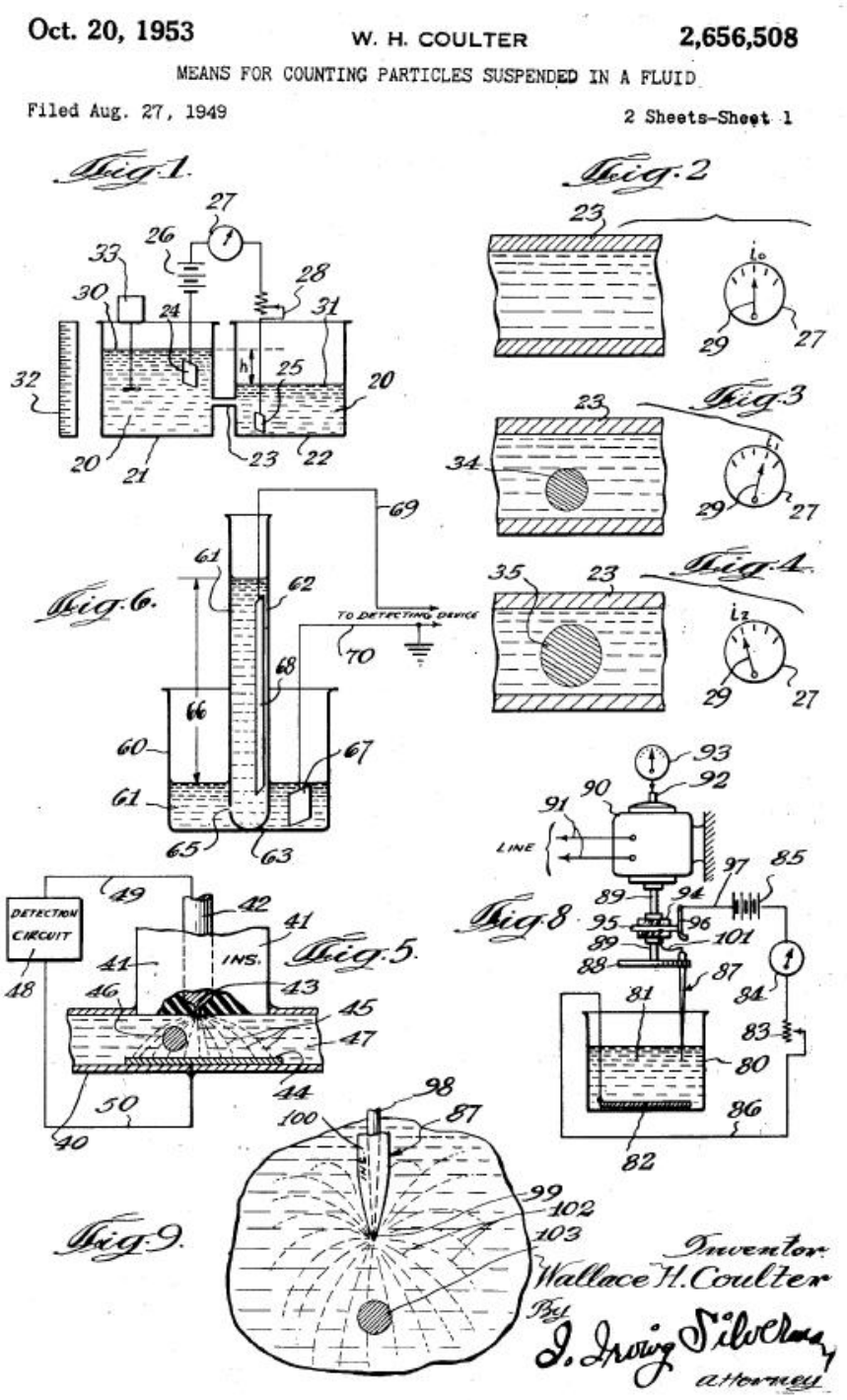
The RBC method



The Coulter principle



Coulter



Oct. 20, 1953 W. H. COULTER 2,656,508

MEANS FOR COUNTING PARTICLES SUSPENDED IN A FLUID

Filed Aug. 27, 1949 2 Sheets-Sheet 1

Fig. 1.

Fig. 2

Fig. 3

Fig. 4.

Fig. 6.

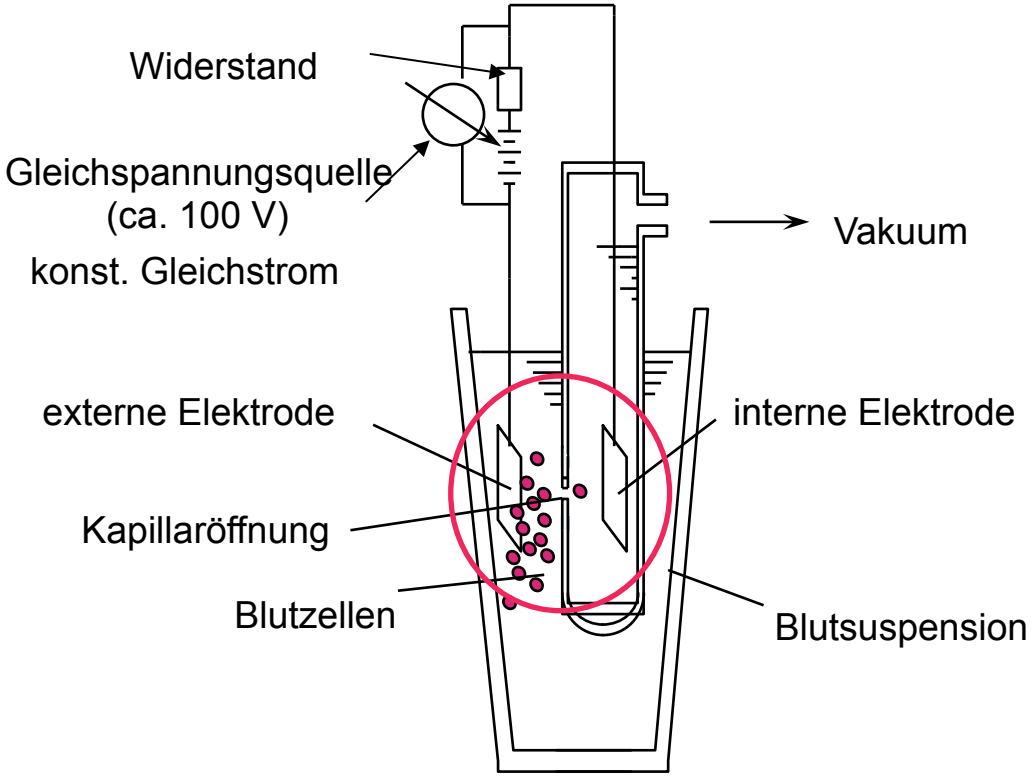
Fig. 5.

Fig. 8

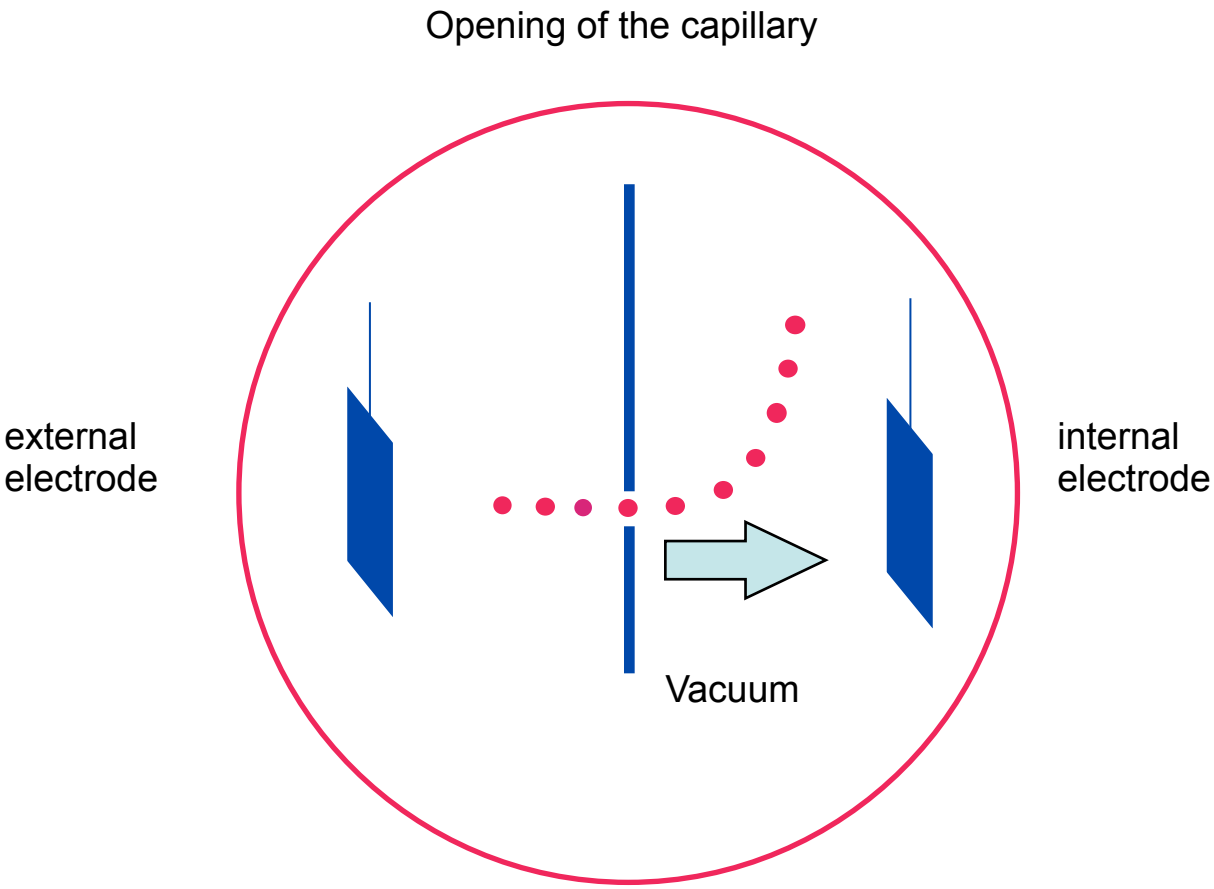
Fig. 9.

Inventor:
Wallace H. Coulter
By J. Irving Silverman
Attorney

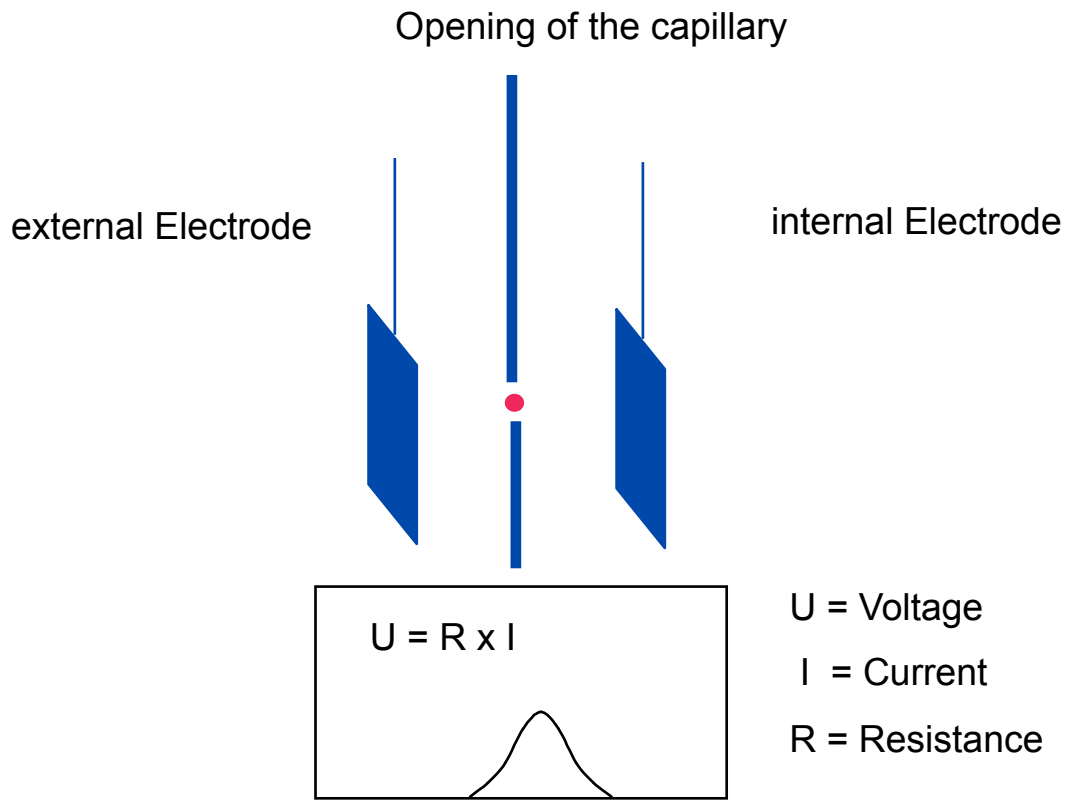
Impedance


Widerstand
Gleichspannungsquelle
(ca. 100 V)
konst. Gleichstrom
Vakuum
externe Elektrode
interne Elektrode
Kapillaröffnung
Blutzellen
Blutsuspension

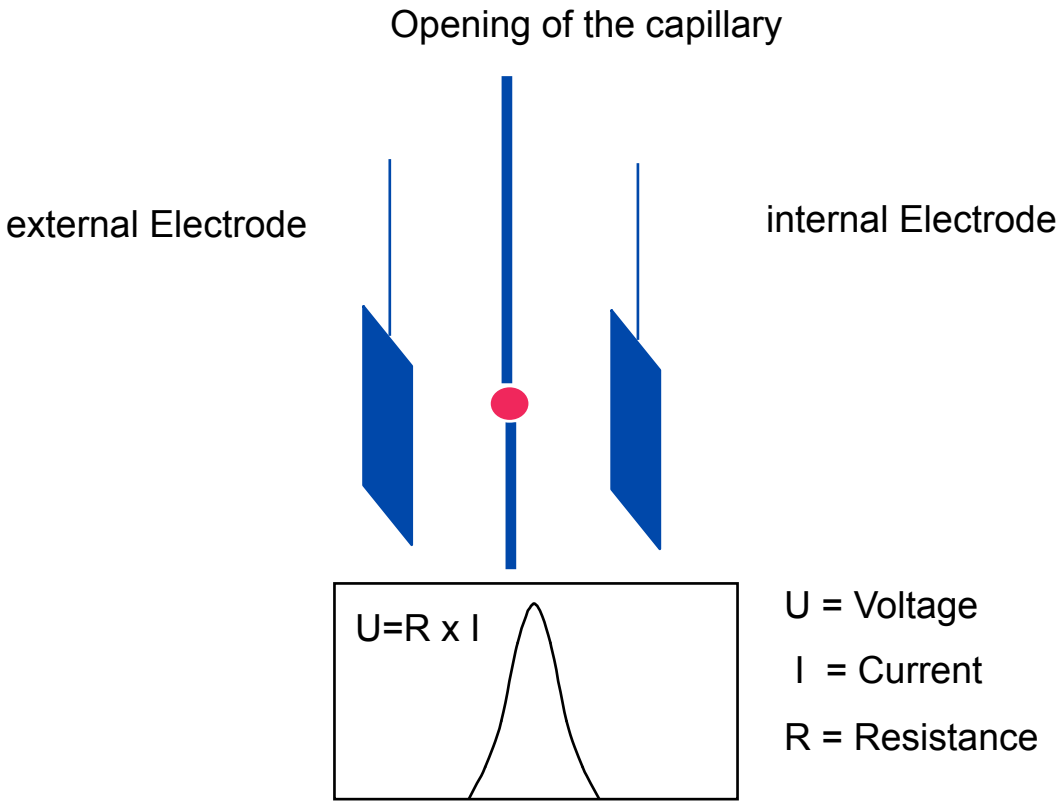
Impedance


Opening of the capillary
external electrode
internal electrode
Vacuum

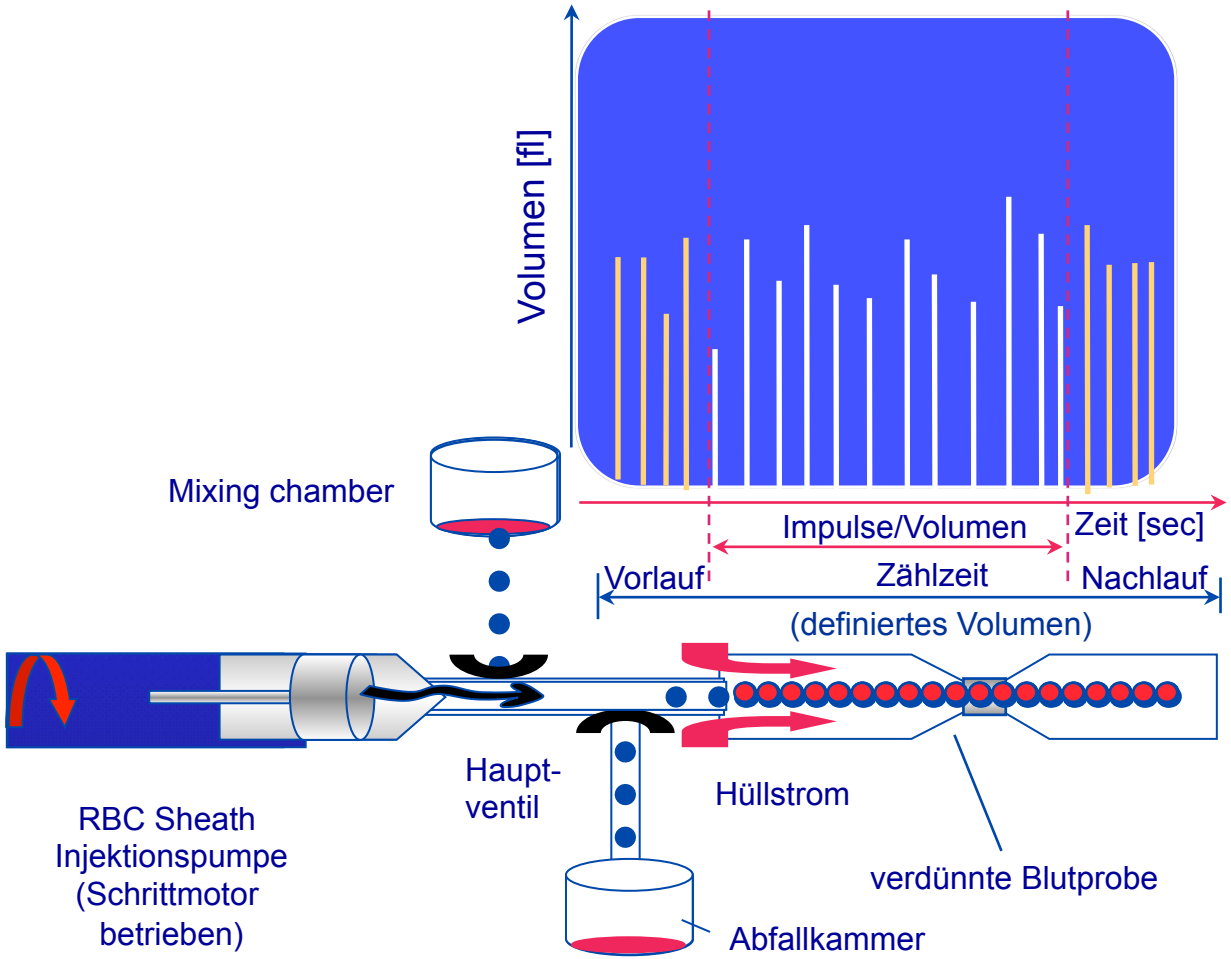
Impedance


Opening of the capillary
external Electrode
internal Electrode
U = R x I
U = Voltage
I = Current
R = Resistance

Impedance


Opening of the capillary
external Electrode
internal Electrode
U=R x I
U = Voltage
I = Current
R = Resistance

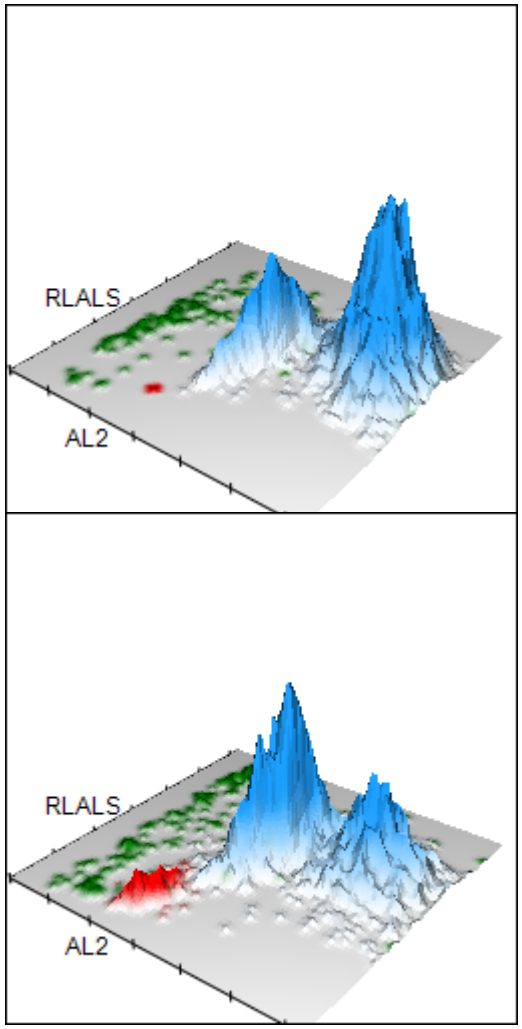
Impedance


Volumen [fl]
Mixing chamber
Impulse/Volumen
Zeit [sec]
Vorlauf
Zählzeit
Nachlauf
(definiertes Volumen)
Haupt-
ventil
Hüllstrom
RBC Sheath
Injektionspumpe
(Schrittmotor
betrieben)
verdünnte Blutprobe
Abfallkammer

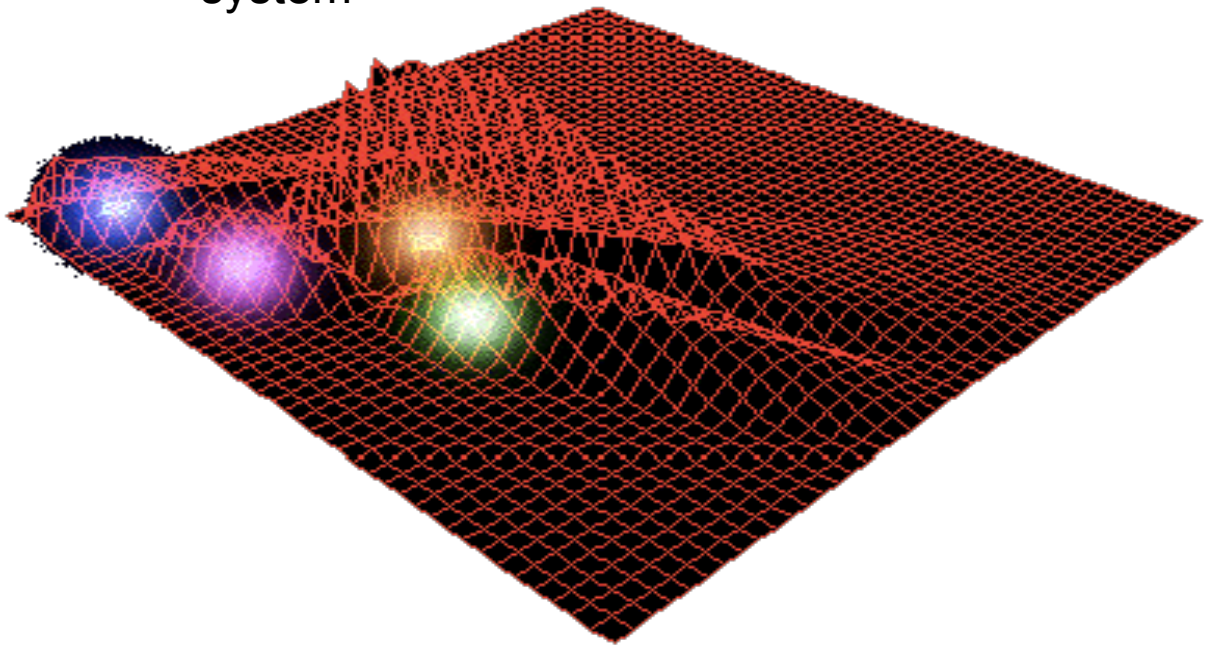
Technologies employed for the differential counts

Instrument and manufacturer	Technology employed for differential count
Coulter	Impedance with low- and high-frequency electromagnetic current Laser light scattering
Sysmex	Impedance with low-frequency direct current Impedance with radiofrequency current
ADVIA	Light scattering Absorbance (Peroxydase)
Cobas	Electrical impedance Light absorbance

Advanced systems



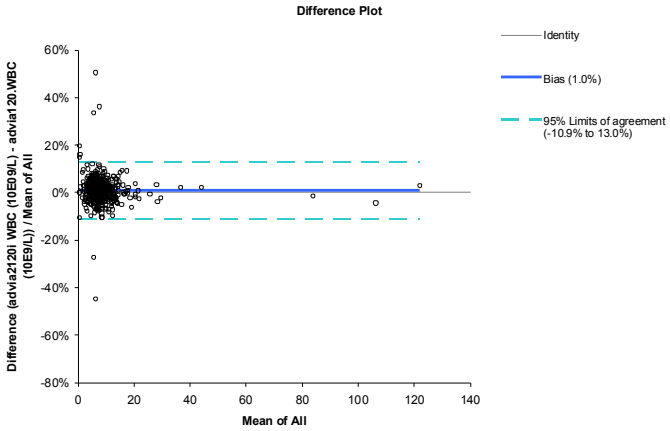
ACAS = Adaptive cluster analysis system



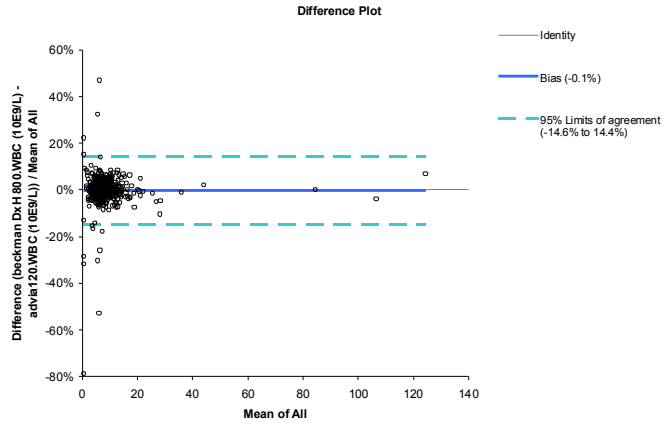
VCS = Volume/Conductivity/Scatter

COMPARABILITY OF THE CELL COUNTERS

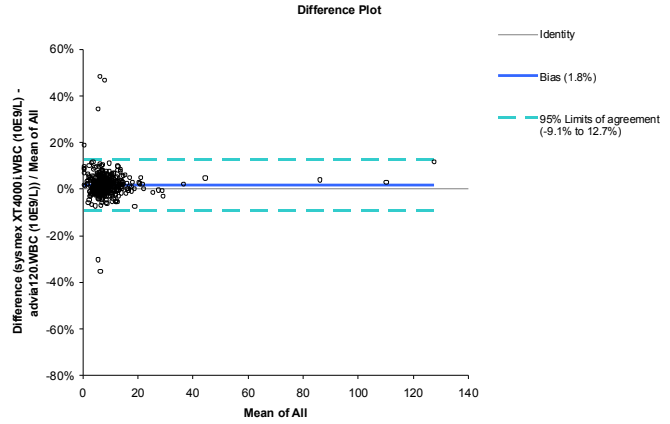
WBC



ADVIA120 vs. ADVIA2120

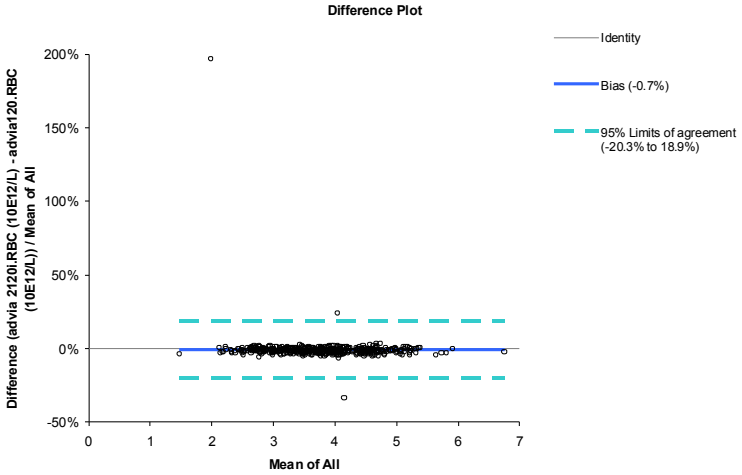


ADVIA120 vs. DxH800

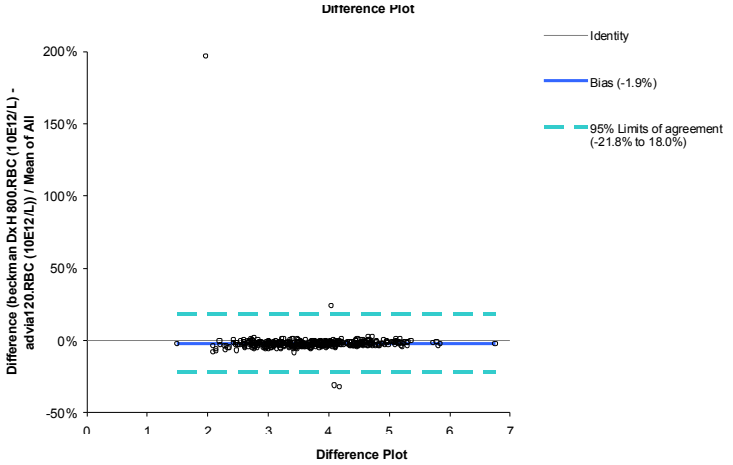


ADVIA120 vs. XE4000i

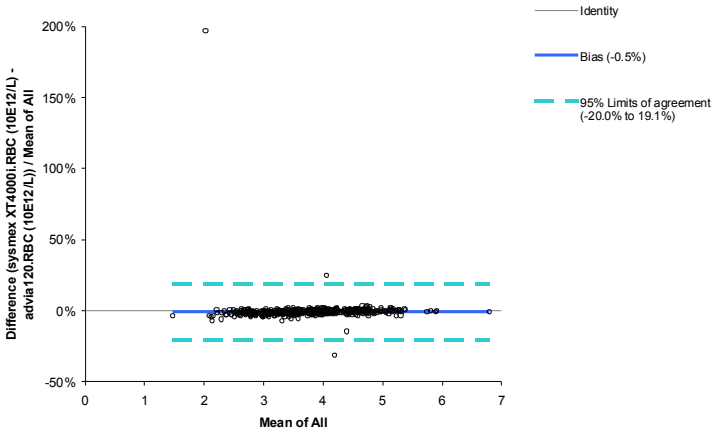
RBC



ADVIA120 vs. ADVIA2120

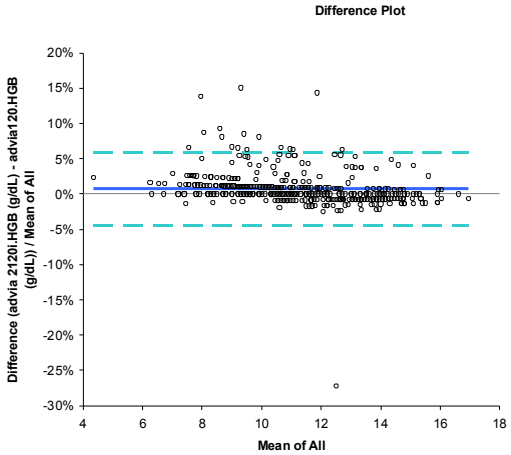


ADVIA120 vs. DxH800

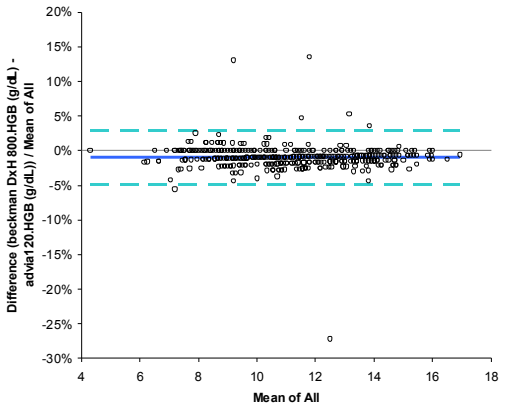


ADVIA120 vs. XE4000i

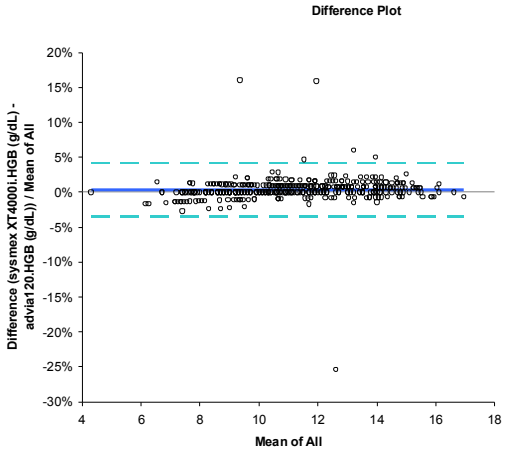
Hemoblobin



ADVIA120 vs. ADVIA2120

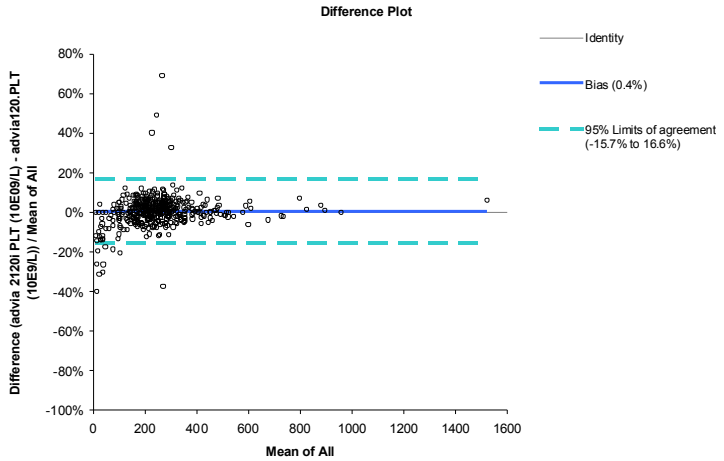


ADVIA120 vs. DxH800

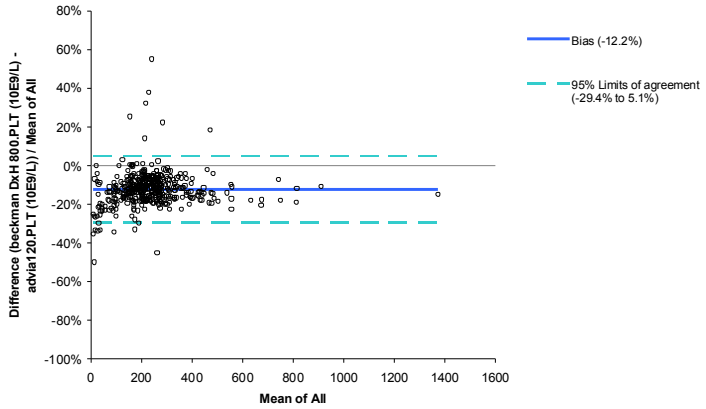


ADVIA120 vs. XE4000i

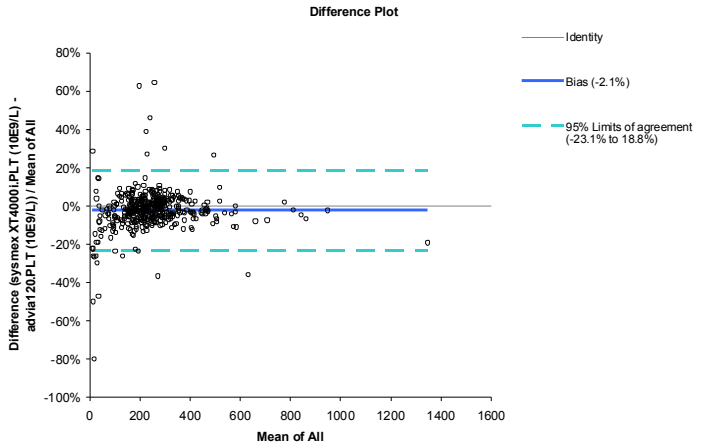
Platelets



ADVIA120 vs. ADVIA2120

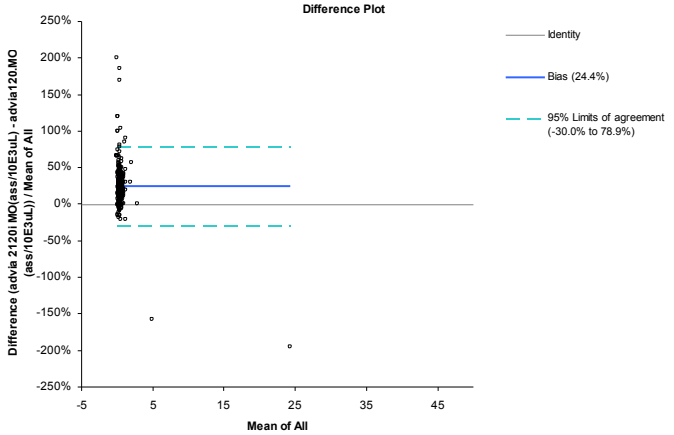


ADVIA120 vs. DxH800

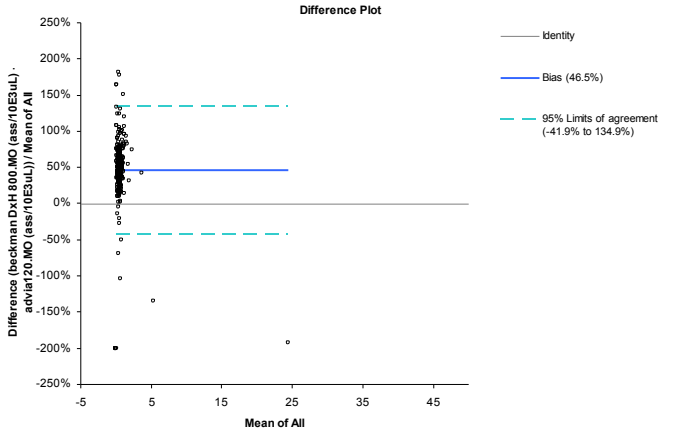


ADVIA120 vs. XE4000i

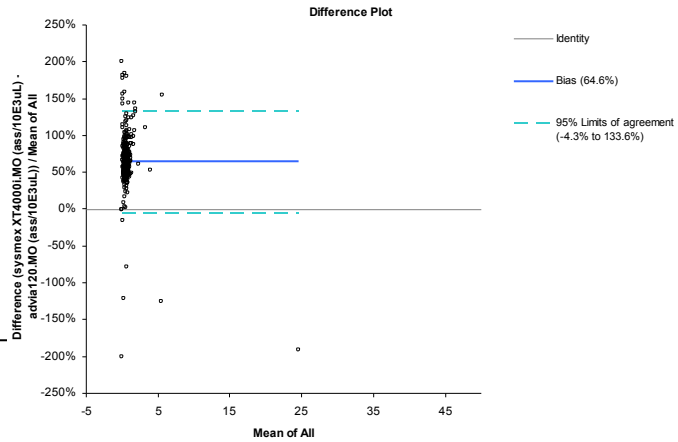
Monocytes



ADVIA120 vs. ADVIA2120



ADVIA120 vs. DxH800



ADVIA120 vs. XE4000i

	CD-Sapphire	ADVIA 120	LH 750	XE-2100
Blasts				
TP	17 (4.0)	13 (3.0)	14 (3.3)	20 (4.7)
TN	391 (90.9)	350 (81.4)	398 (92.6)	369 (85.8)
FP	17 (4.0)	58 (13.5)	10 (2.3)	39 (9.1)
FN	5 (1.2)	9 (2.1)	8 (1.9)	2 (0.5)
Sensitivity (%)	77.3	59.1*	63.6	90.9*
Specificity (%)	95.8	85.8	97.5	90.4
Efficiency (%)	94.9	84.4†	95.8†	90.5
Immature granulocytes and/or Left shift				
TP	59 (13.7)	51 (11.9)	36 (8.4)	56 (13.0)
TN	303 (70.5)	274 (63.7)	346 (80.5)	328 (76.3)
FP	58 (13.5)	87 (20.2)	15 (3.5)	33 (7.7)
FN	10 (2.3)	18 (4.2)	33 (7.7)	13 (3.0)
Sensitivity (%)	85.5‡	73.9	52.2‡	81.2
Specificity (%)	83.9	75.9	95.8	90.9
Efficiency (%)	94.2§	75.6§	88.8	89.3
Atypical lymphocytes				
TP	2 (0.5)	3 (0.7)	0 (0.0)	2 (0.5)
TN	387 (90.0)	362 (84.2)	407 (94.7)	381 (88.6)
FP	38 (8.8)	63 (14.7)	18 (4.2)	44 (10.2)
FN	3 (0.7)	2 (0.5)	5 (1.2)	3 (0.7)
Sensitivity (%)	40.0	60.0	0.0	40.0
Specificity (%)	91.1	85.2	95.8	89.6
Efficiency (%)	90.5	84.9¶	94.7¶	89.1
Nucleated RBC				
TP	4 (0.9)	2 (0.5)	9 (2.1)	7 (1.6)
TN	411 (95.6)	407 (94.7)	403 (93.7)	400 (93.0)
FP	7 (1.6)	11 (2.6)	15 (3.5)	18 (4.2)
FN	8 (1.9)	10 (2.3)	3 (0.7)	5 (1.2)
Sensitivity (%)	33.3	16.7**	75.0**	58.3
Specificity (%)	98.3	97.4	96.4	95.7
Efficiency (%)	96.5	95.1	95.8	94.7

KANG SH, KIM HK, HAM CK, LEE DS, CHO HI. Comparison of four hematology analyzers, CELL-DYN Sapphire, ADVIA 120, Coulter LH 750, and Sysmex XE-2100, in terms of clinical usefulness. *Int J Lab Hematol*. 2008;30(6):480–486.

NEW PARAMETERS IN BLOOD CELL COUNTING

Newer parameters

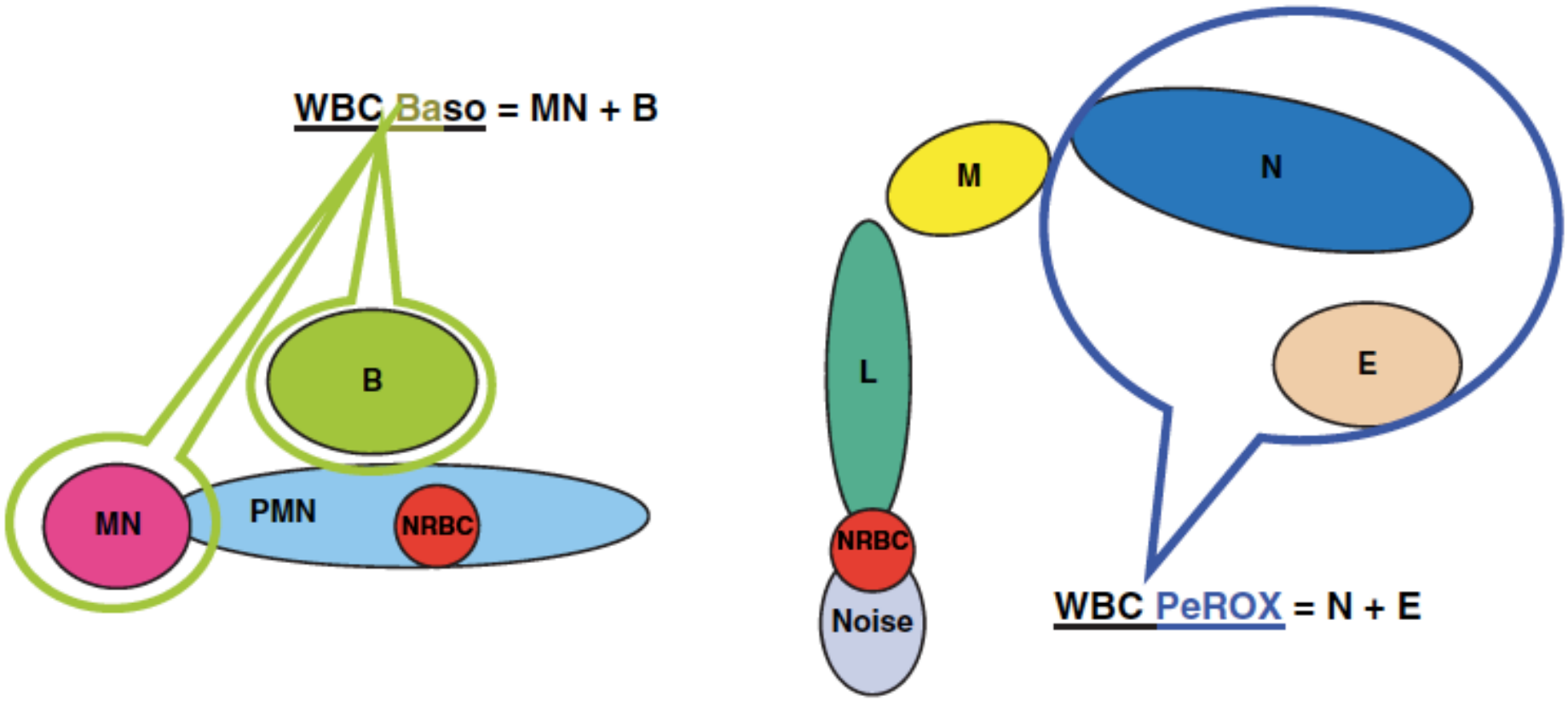
	DxH800	XE4000i	ADVIA 2120
Nucleated RBC (NRBC)	✓	✓	✓
Immature granulocytes (IG)		✓	(✓)
Reticulocyte hemoglobin	(✓)	(✓)	✓
Reticulocyte volume (MRV)	✓		
Immature reticulocytes (IR)	✓	✓	✓
Hypochromic RBC	(✓)	✓	✓
Fragmented red cells (FRC)		✓	✓
Immature platelet fraction (IPF)		✓	

Reticulocyte hemoglobin DxH800: RSF, XE4000i Ret-Hb, ADVIA2120 CHr
Hypochromic RBC: DxH800: LHD, ADVIA2120 Hypochromic RBC

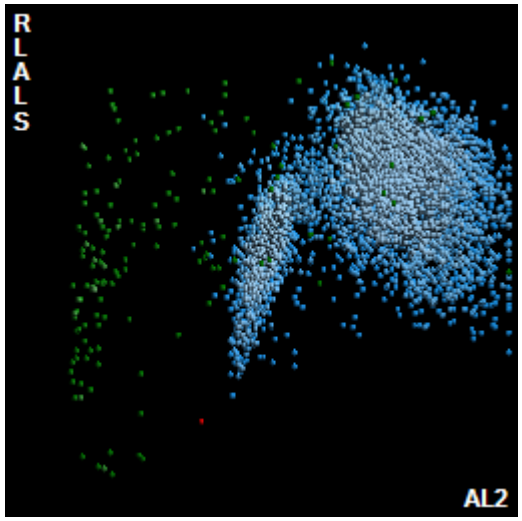
Nucleated red blood cells

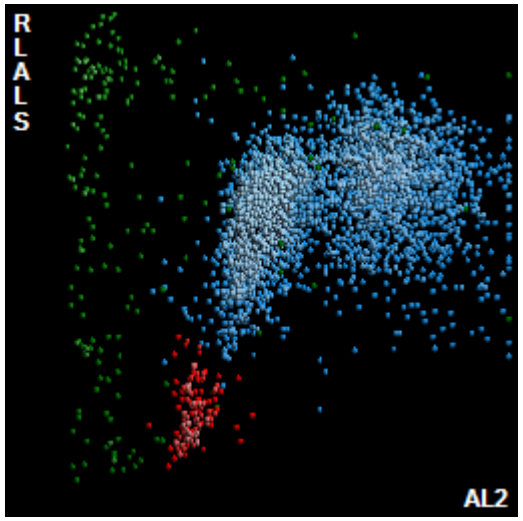
- Clinical significance
  - Disease detection
  - Automatic correction of WBC and lymphocyte counts
- Different techniques
  - Signature position on the impedance size WBC histogram (DxH800)
  - Basophil channel and the Peroxidase channel (ADVIA)
  - Flow cytometry (XE-2100)

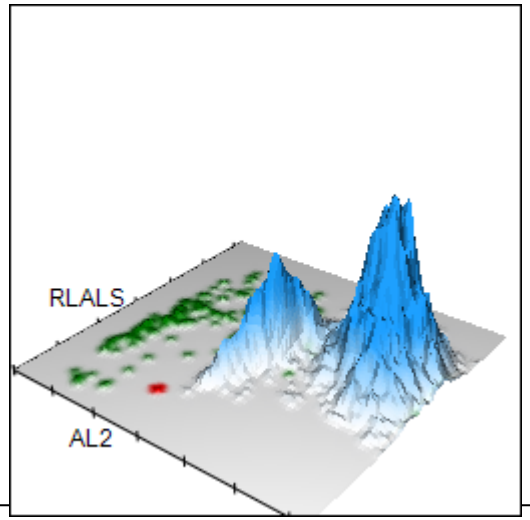
NRBC ADVIA

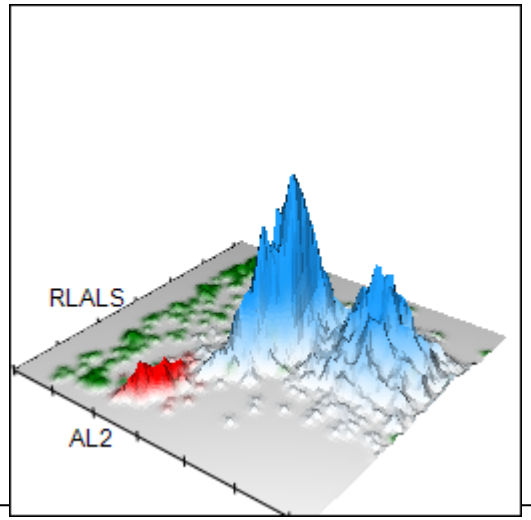


NRBC Beckman Coulter





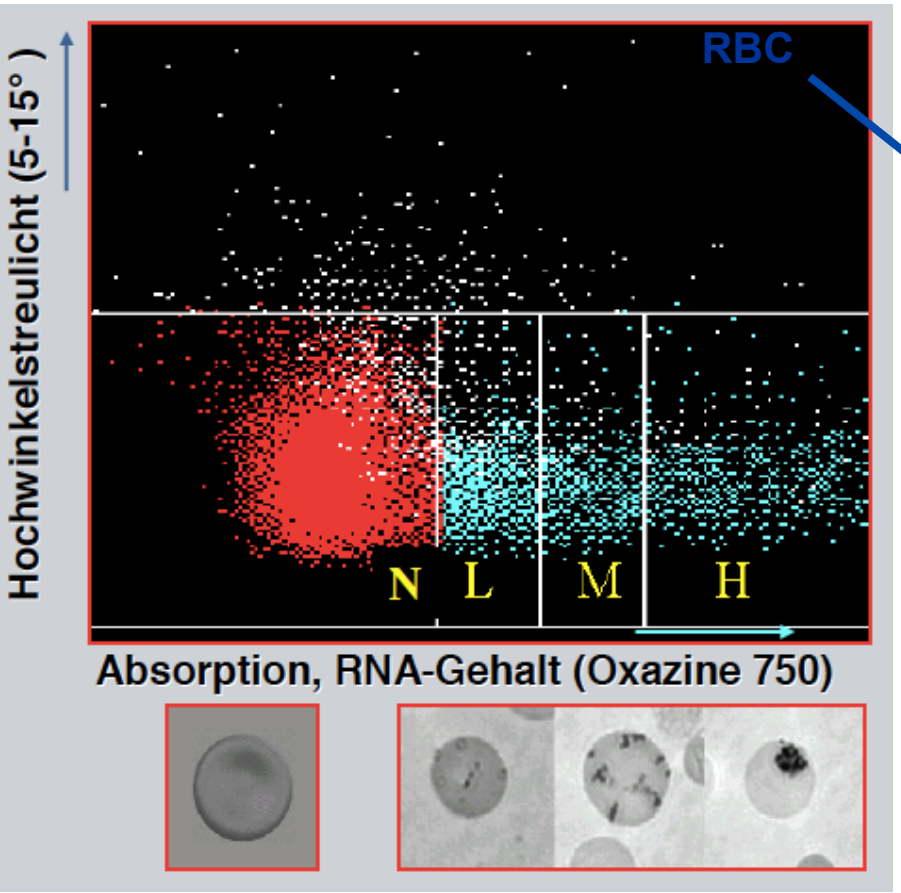


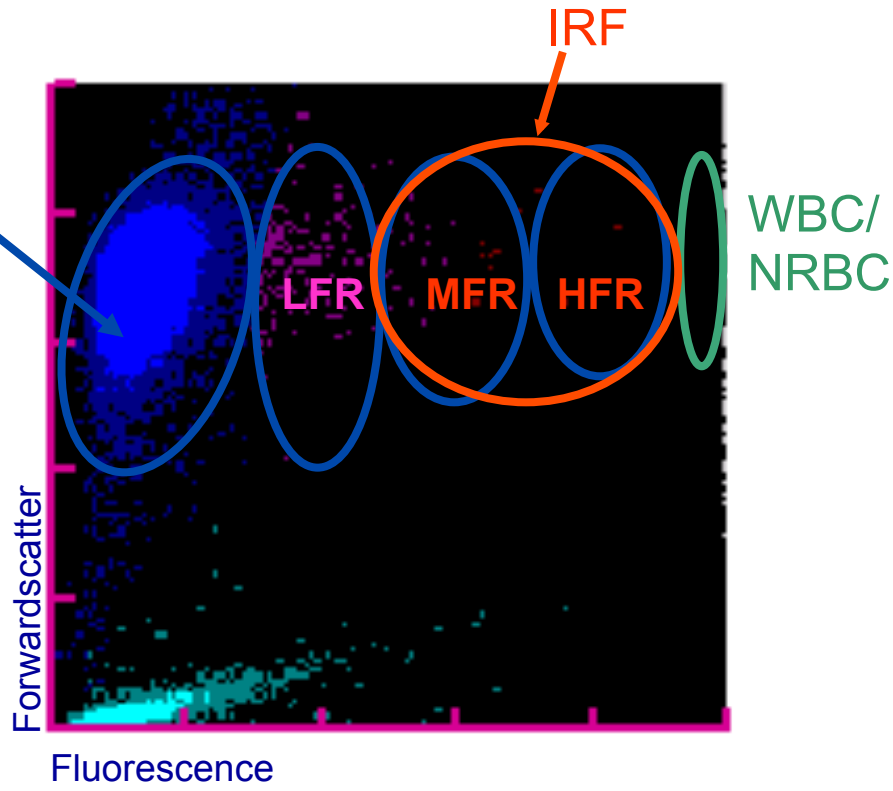


Immature reticulocyte fraction (IRF)

- Most immature reticulocytes
- Clinical significance
  - Earliest increase of the reticulocyte fraction
  - Monitoring BM regeneration
  - Hemolysis
  - Therapy of iron-deficiency

Immature reticulocyte fraction

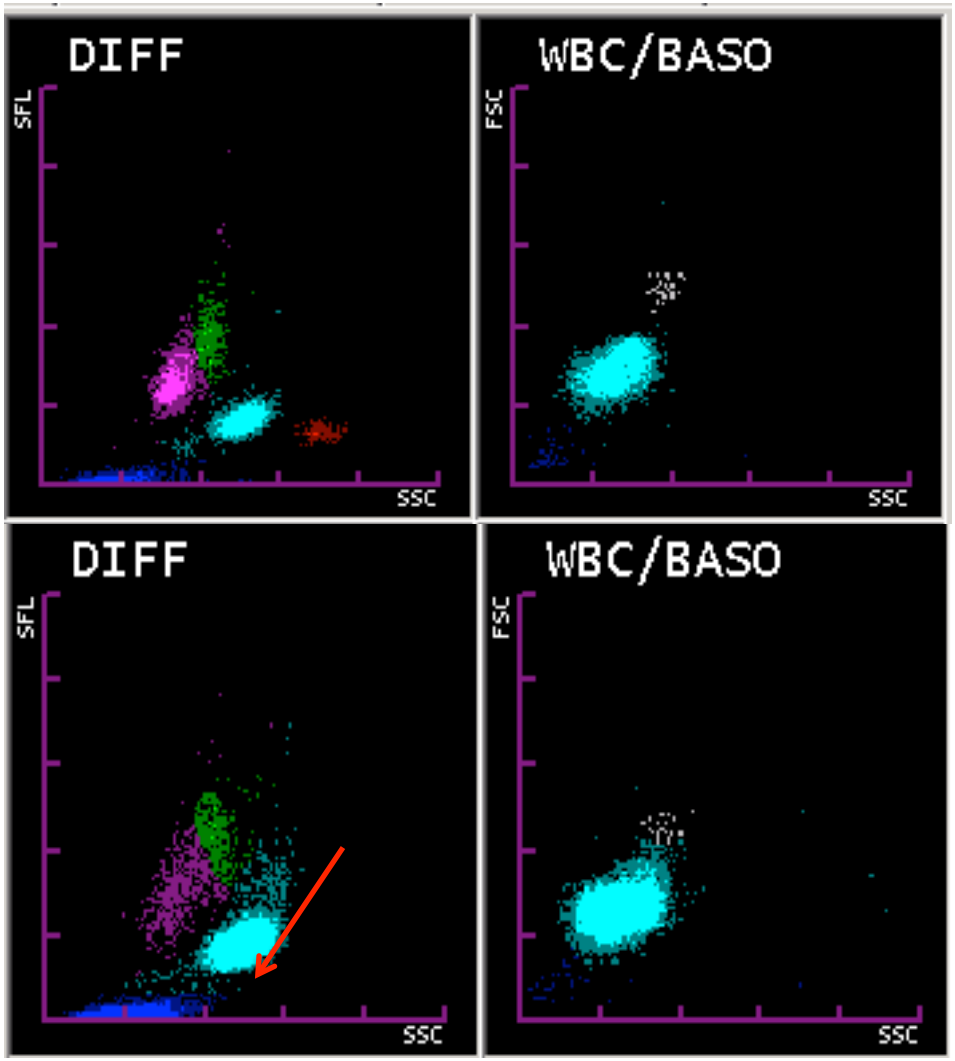




Parameters of functional iron deficiency

- **CHr**, hypochromic RBC (ADVIA)
  - Measurement: Two-angle scatter
- **Ret-He** (Sysmex)
  - Reticulocyte fluorescence and FSC
- **MRV** (mean reticulocyte volume), **LHD** (Low hemoglobin density), **MAF** (microcytic anemia factor, **RSf** (Red blood cell size factor), (Beckman Coulter)
- Clinical utility
  - Predicting of iron deficiency
  - Manage iron supplementation therapy

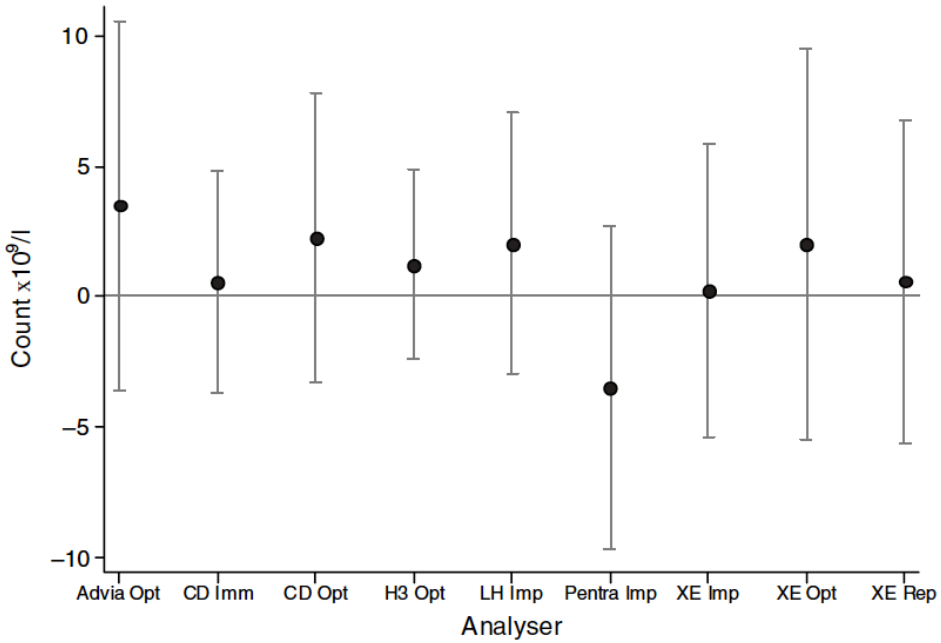
Immature granulocytes

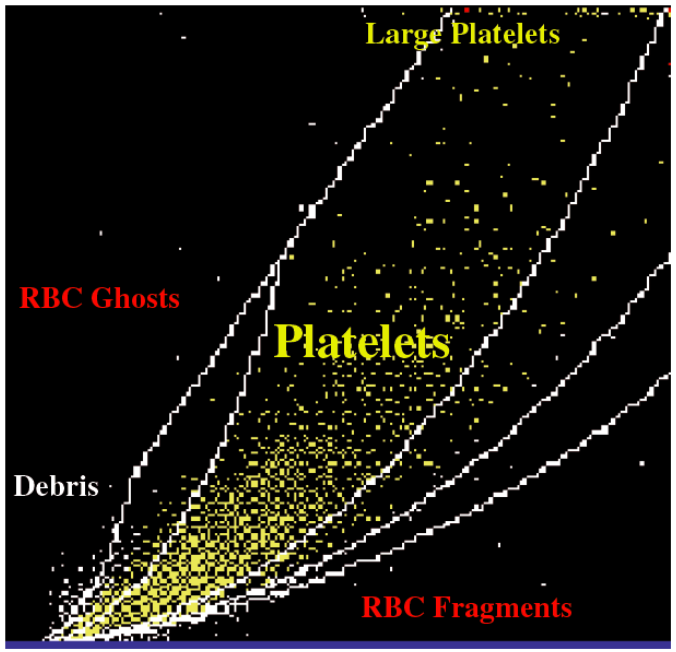


Immature granulocytes

- Sysmex only
- Promyelocytes, myelocytes, metamyelocytes
- Clinical application??
  - Detection of inflammation

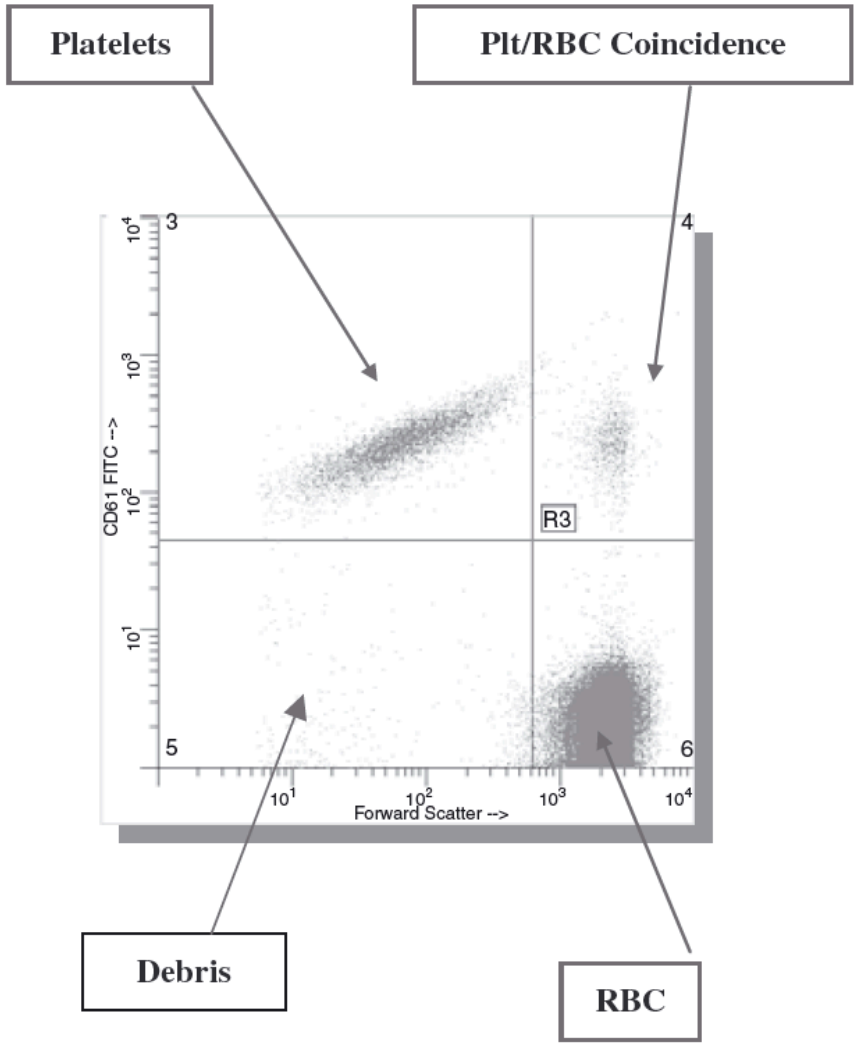
Platelet measurement



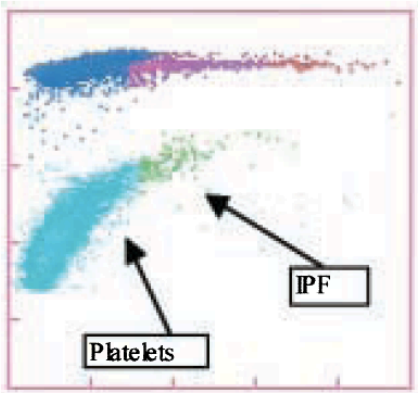
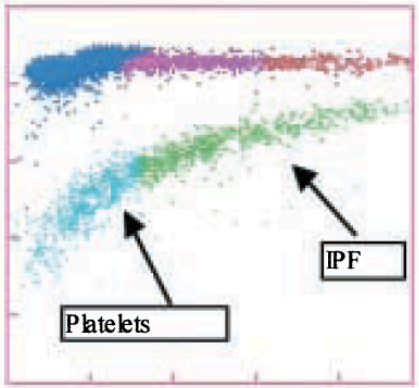


Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count. *Int J Lab Hematol*. 2007;29(2):77–91.
Segal HC, Briggs C, Kunka S, et al. Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. *Br J Haematol*. 2005;128(4):520–525.

Immunological platelet counting

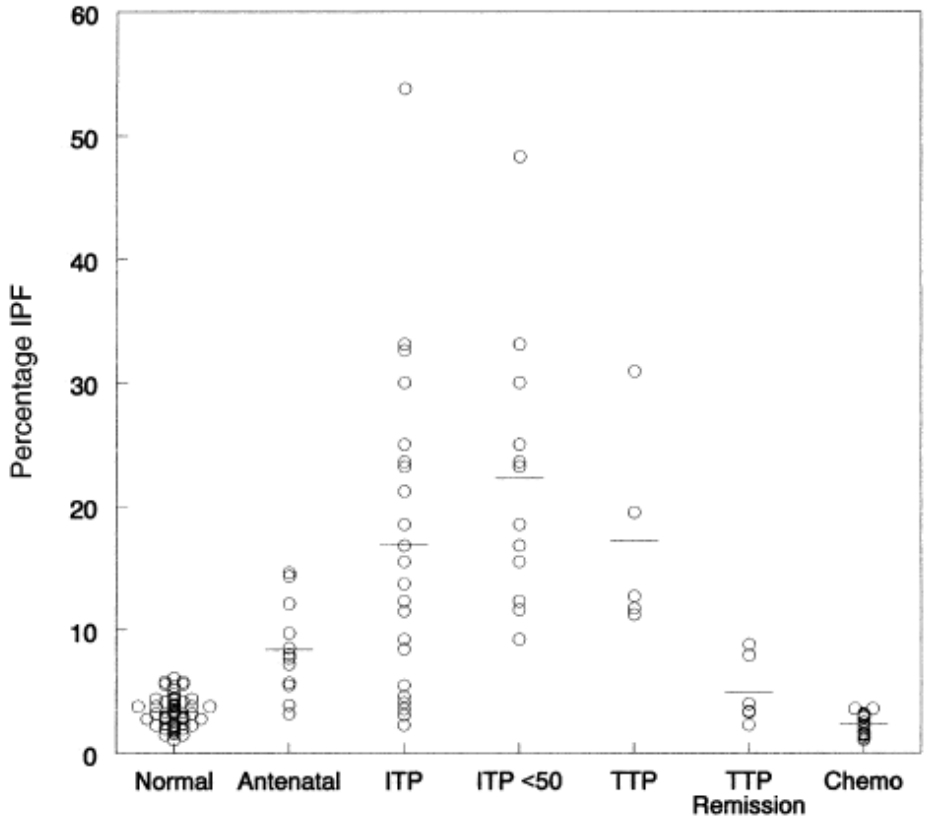


Immature platelet fraction

Normal	ITP/PREG
Platelet count 227 ($x10^9$/l) **IPF 3·4%** 	**Platelet count** 74 ($x10^9$/l) **IPF 53·8%** 

Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. *Br J Haematol*. 2004;126(1):93–99.

Immature platelet fraction

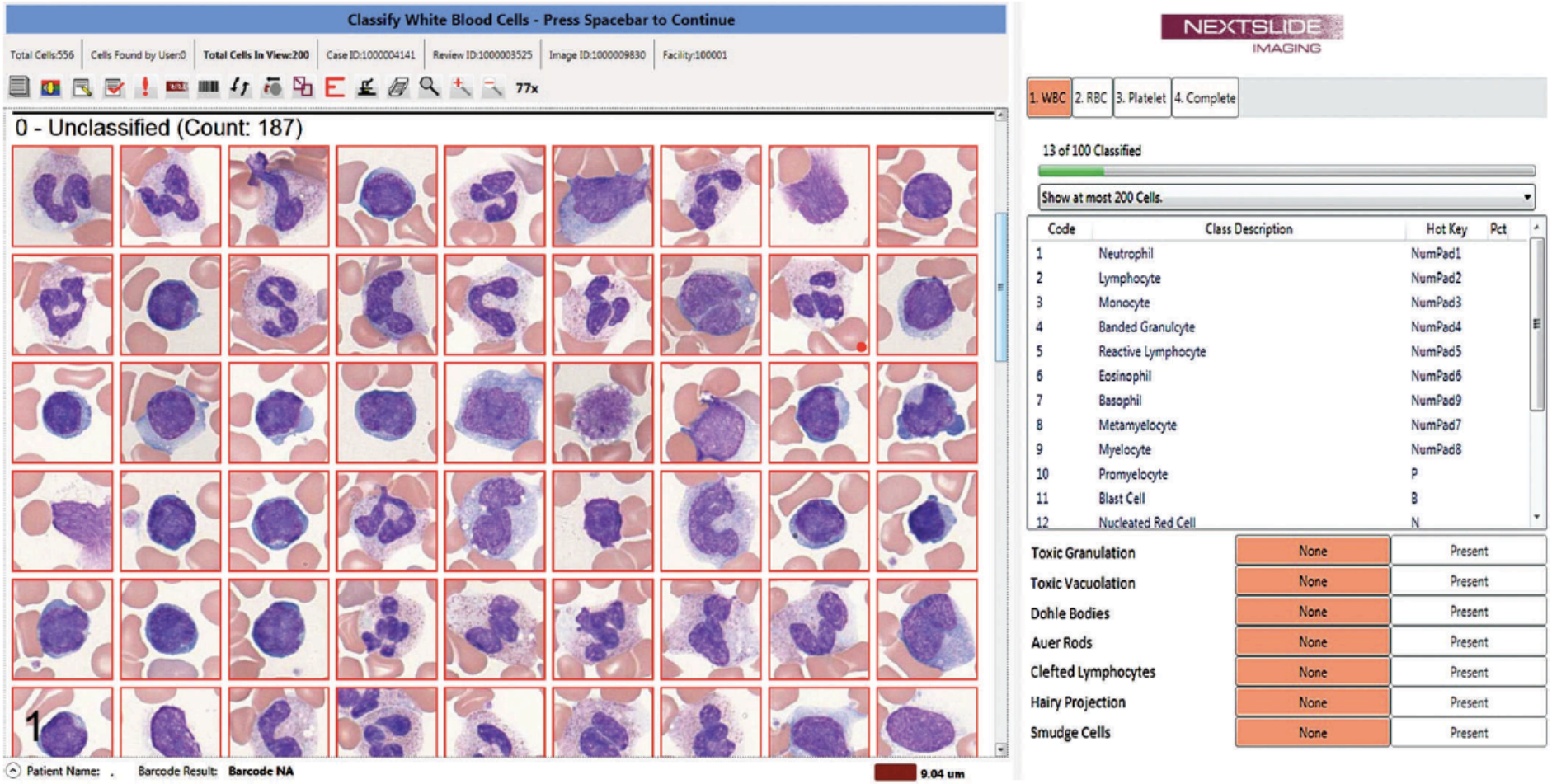


Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. *Br J Haematol*. 2004;126(1):93–99.

Conclusions

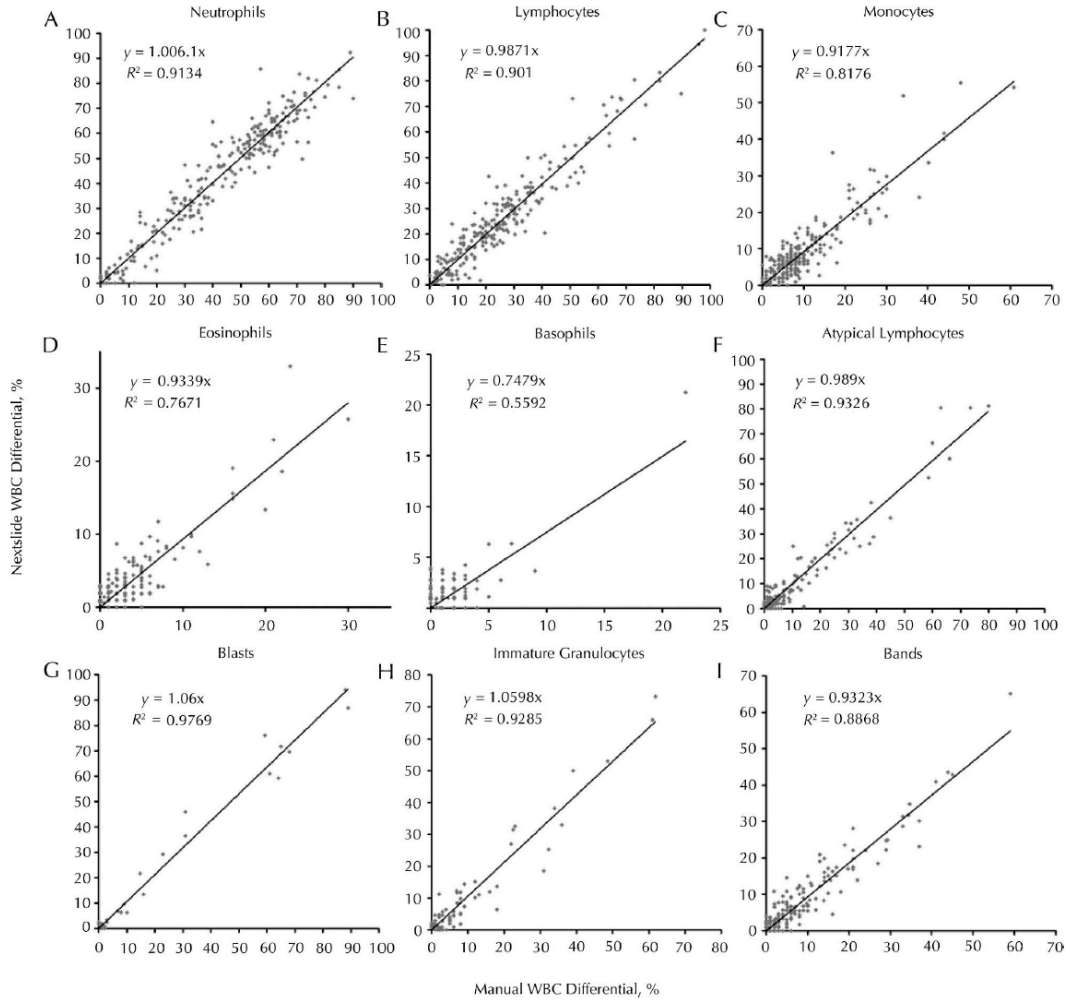
- Cell counters are increasingly sophisticated
- The basic values are comparable
- Increasing amount of new parameters
  - Often no internal or external QC
- The complex analysis matrices of the new cell counters offer interesting options
- Their clinical applications have to be evaluated

Automated digital imaging systems



Yu H, Ok CY, Hesse A, et al. Evaluation of an automated digital imaging system, nextslide digital review network, for examination of peripheral blood smears. *Arch Pathol Lab Med*. 2012;136(6):660–667.

Automated digital imaging systems



Yu H, Ok CY, Hesse A, et al. Evaluation of an automated digital imaging system, nextslide digital review network, for examination of peripheral blood smears. *Arch Pathol Lab Med*. 2012;136(6):660–667.

Is there still a role for manual blood count?

Yes, there is, but

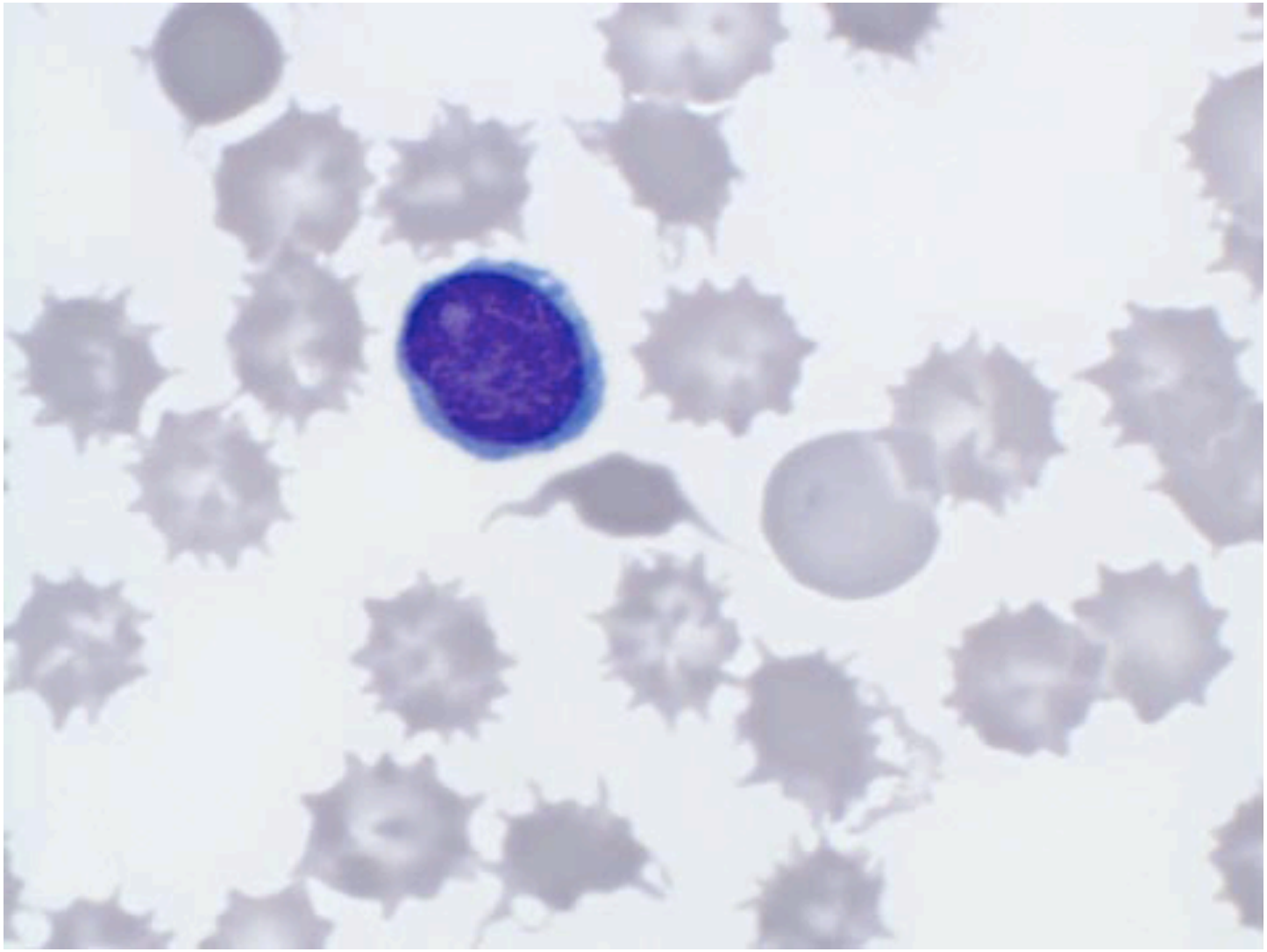
qualitative, not quantitative

Manual vs automated: RBC

	Automated	Manual
Hemoglobin	+++	-
Micro-/Macrocytosis	+++	-
Hypo-/Hyperchromia	+++	-
Anisocytosis	+++	+
Fragmentocytes	+	+++
Poikilocytosis	-	+++
RBC inclusions	-	+++
Reticulocytes	+++	+ (Supravital stainings)
Dysplasia	+	+++

Manual vs automated: WBC

	Automated	Manual
Neutrophils	++	++
Basophils	+++ (if neg), - (if pos)	+++
Lymphocytes	++	++
Eosinophils	+++	++
Monocytes	+	++
Myeloid precursors	+	+++
Blasts	+++	+++
…		

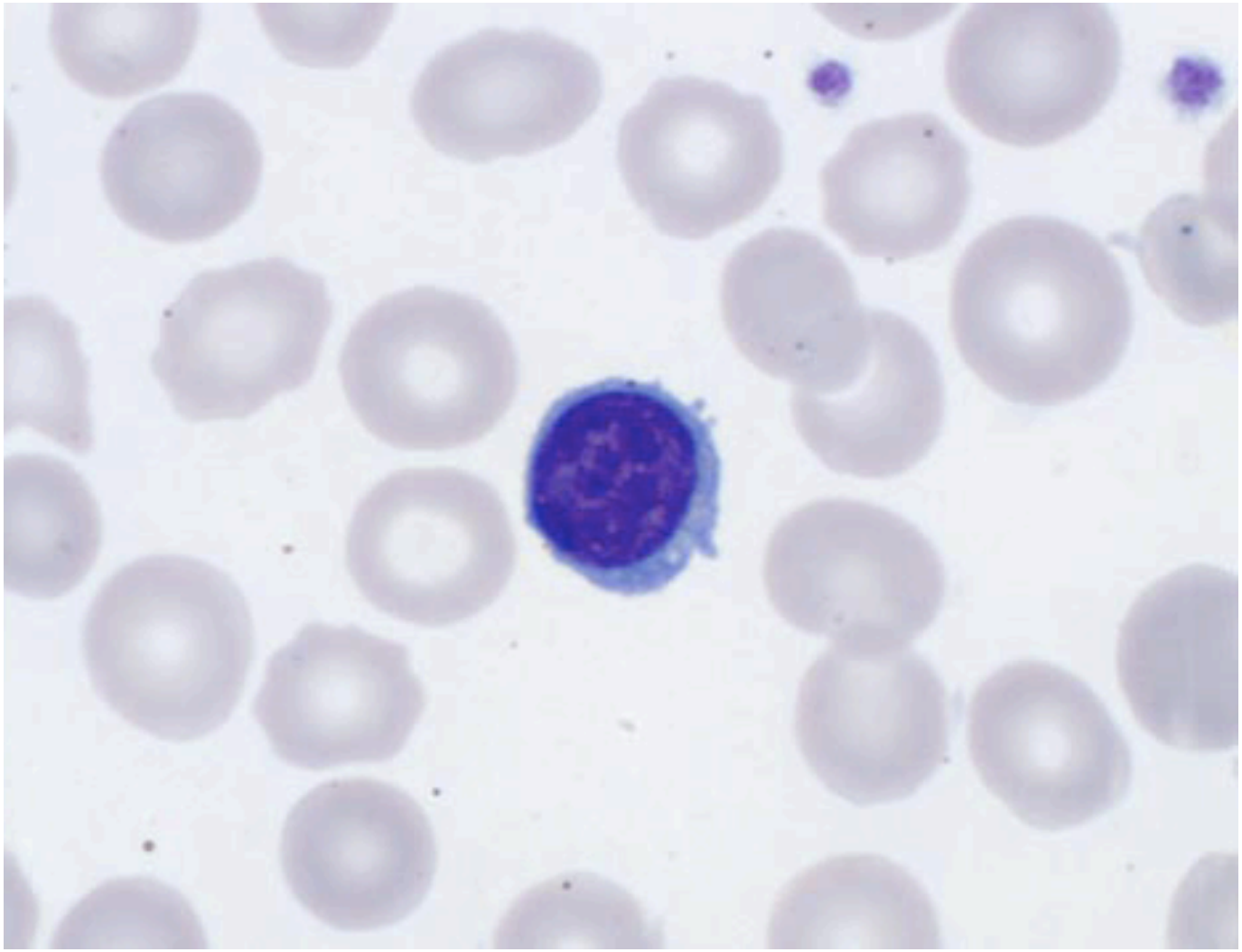


Gerber, Blood, 2011

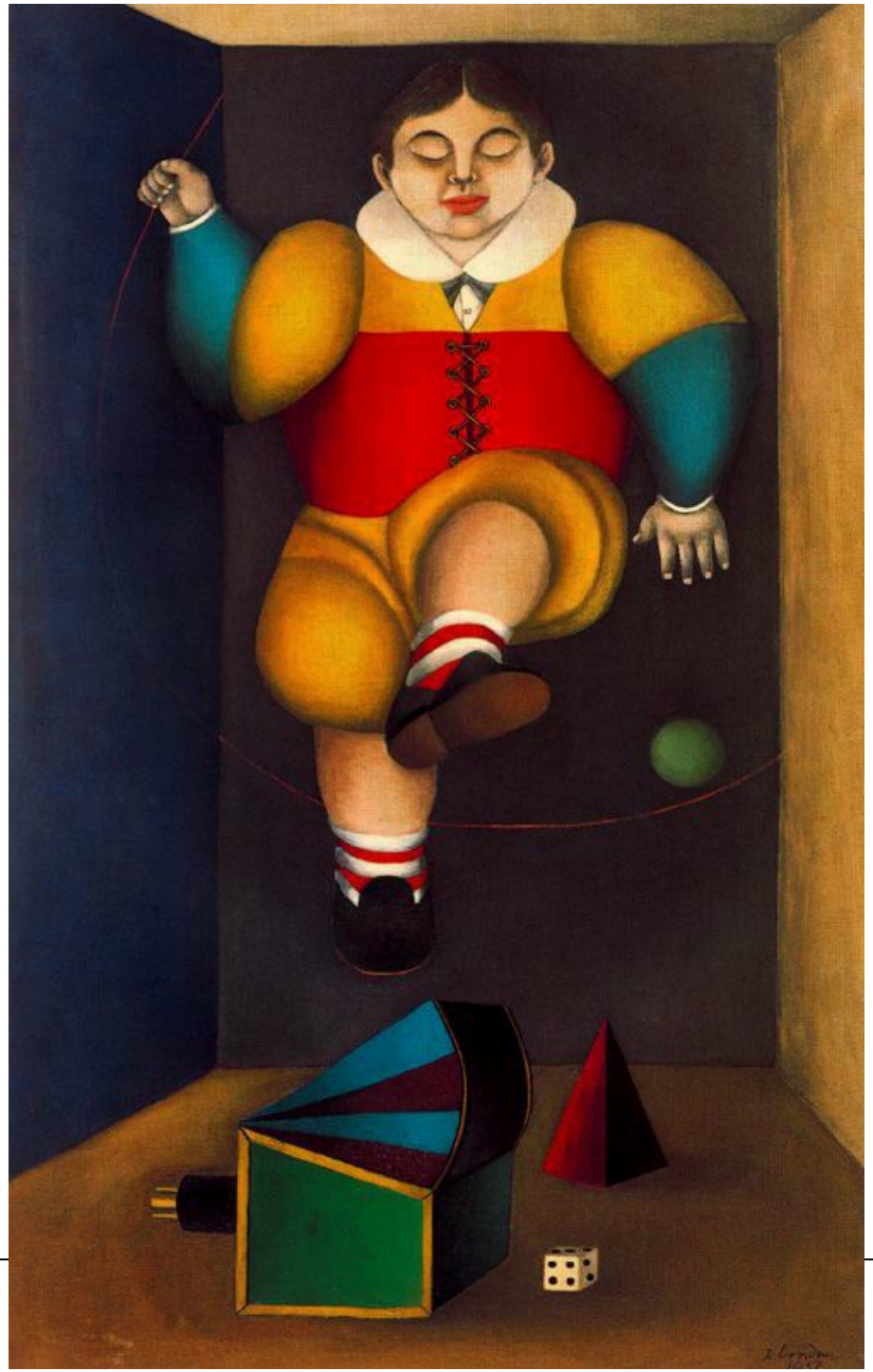
Case report

- A 34-year-old patient
- Coombs negative hemolytic anemia with numerous spiculated RBC
- Spur cell anemia
  - end-stage alcoholic liver disease, renal failure
  - accumulation of excess membrane cholesterol
  - impaired deformability and reduced RBC survival
  - Poor prognosis (median survival of a few months)

- Orthotopic liver transplantation
  - Primary graft failure
- Urgent retransplantation
- 3 months follow-up:
  - Alcohol abstinence
  - Sustained improvement of the anemia and transfusion independence.



Gerber, Blood, 2011



Automated vs manual

Automated analyzers

Pro

- Superior accuracy and precision
- Fast
- Online possibilities

Con

- Complexity and variation of cells remains challenging for analyzers
- Acquisition costs

Manual evaluation

Pro

- Overview
- Dysplatic changes
- Long experience

Con

- Labor-intensive
- Expensive
- Requires highly-trained stuff
- Delayed results