Can I Diagnose Cancer With My Haematology Analyzer?

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Disclaimer

* I am an internist and oncologist, not a clinical pathologist
* I consult with Idexx
* I have used Idexx equipment for over 20 years
* This lecture is sponsored by IDEXX Laboratories, Inc

Hematology In Cancer Patients

- Anemia
- Neutropenia
- Neutrophilia
- Left shift / toxicity
- Lymphoma
- Leukemia
- Thrombocytopenia
- Serial data analysis during chemotherapy

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This Lecture

- Analyzers
- Lymphomas
- Leukemias

In-House Hematology Analyzers

pre1980s — 1980s — 1990s — 2003 — 2010 — 2021

Manual

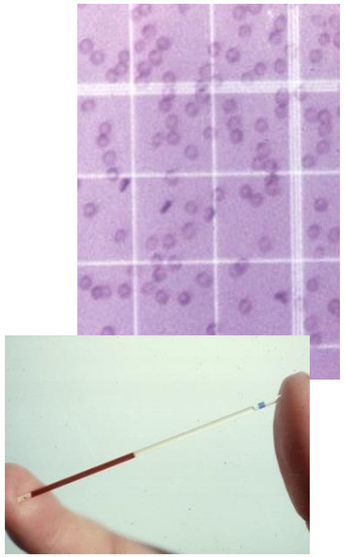
Quantitative Buffy coat (QBC)

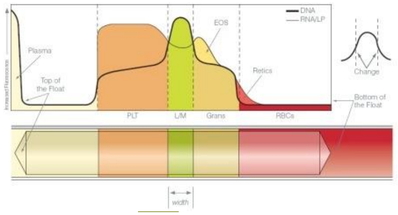
Impedance

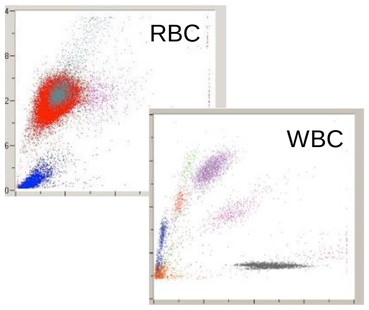
LaserCyte

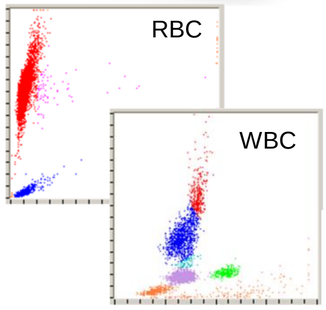
ProCyte Dx

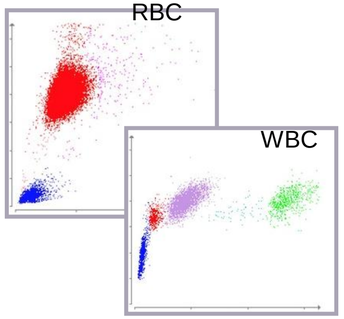
ProCyte One





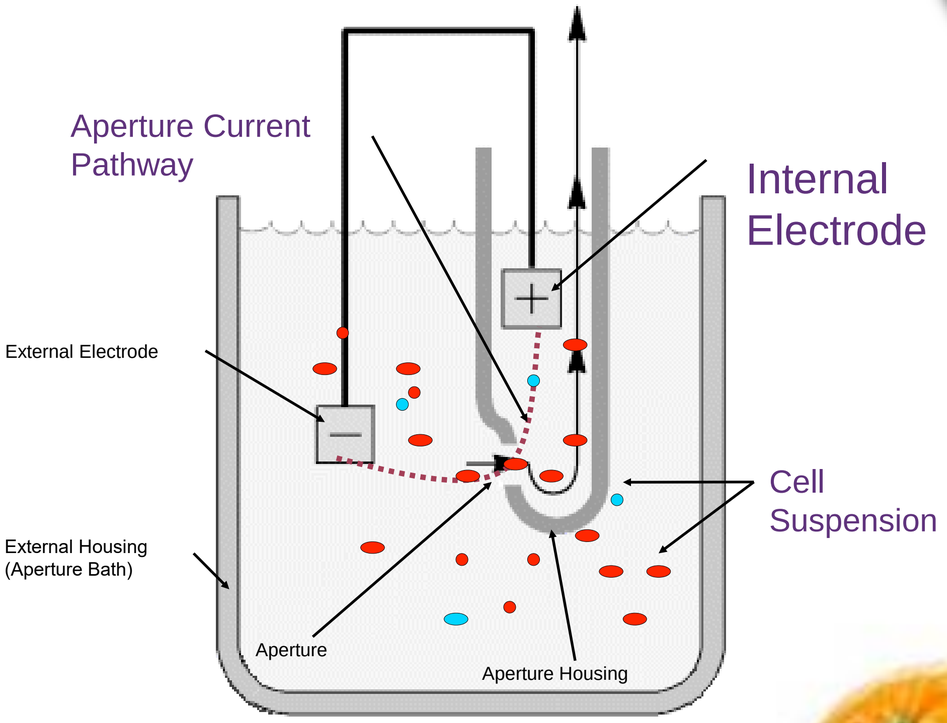


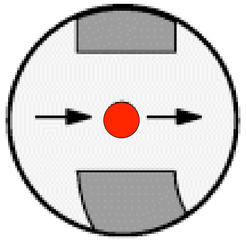




Impedance

Change in resistance is proportional to cell volume

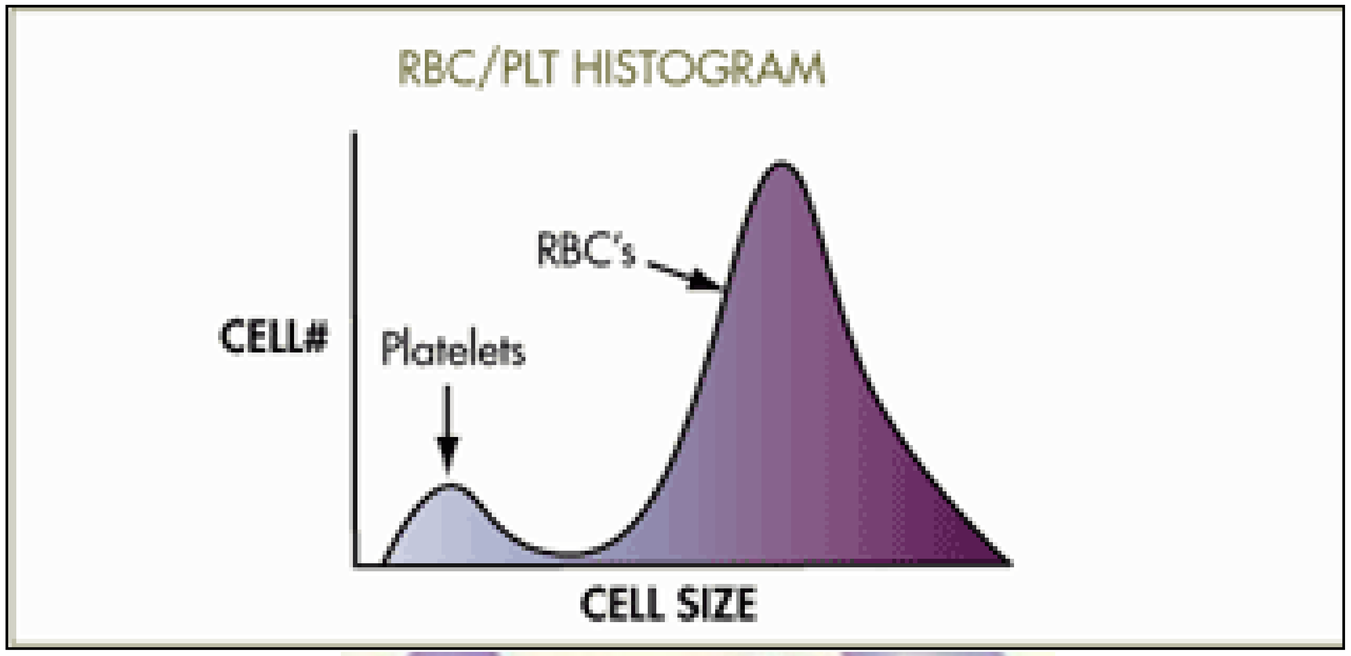


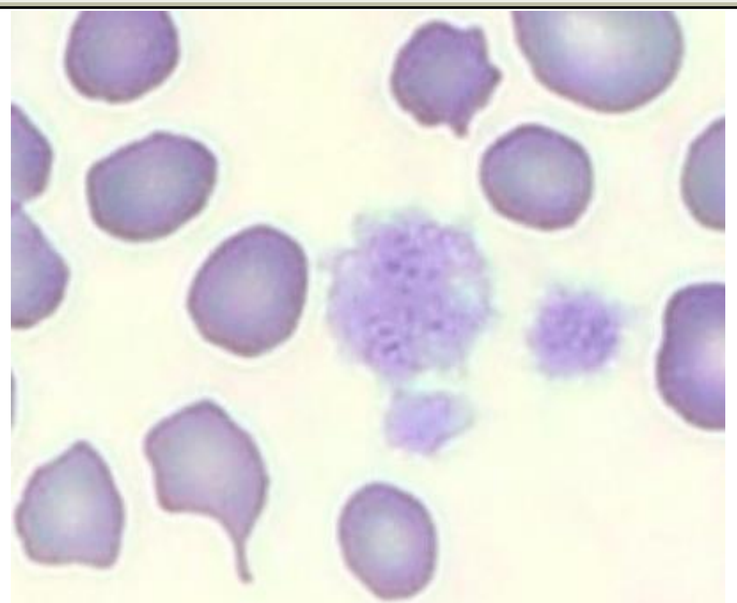


Detail of Aperture

Courtesy Dr. Bill Saxon

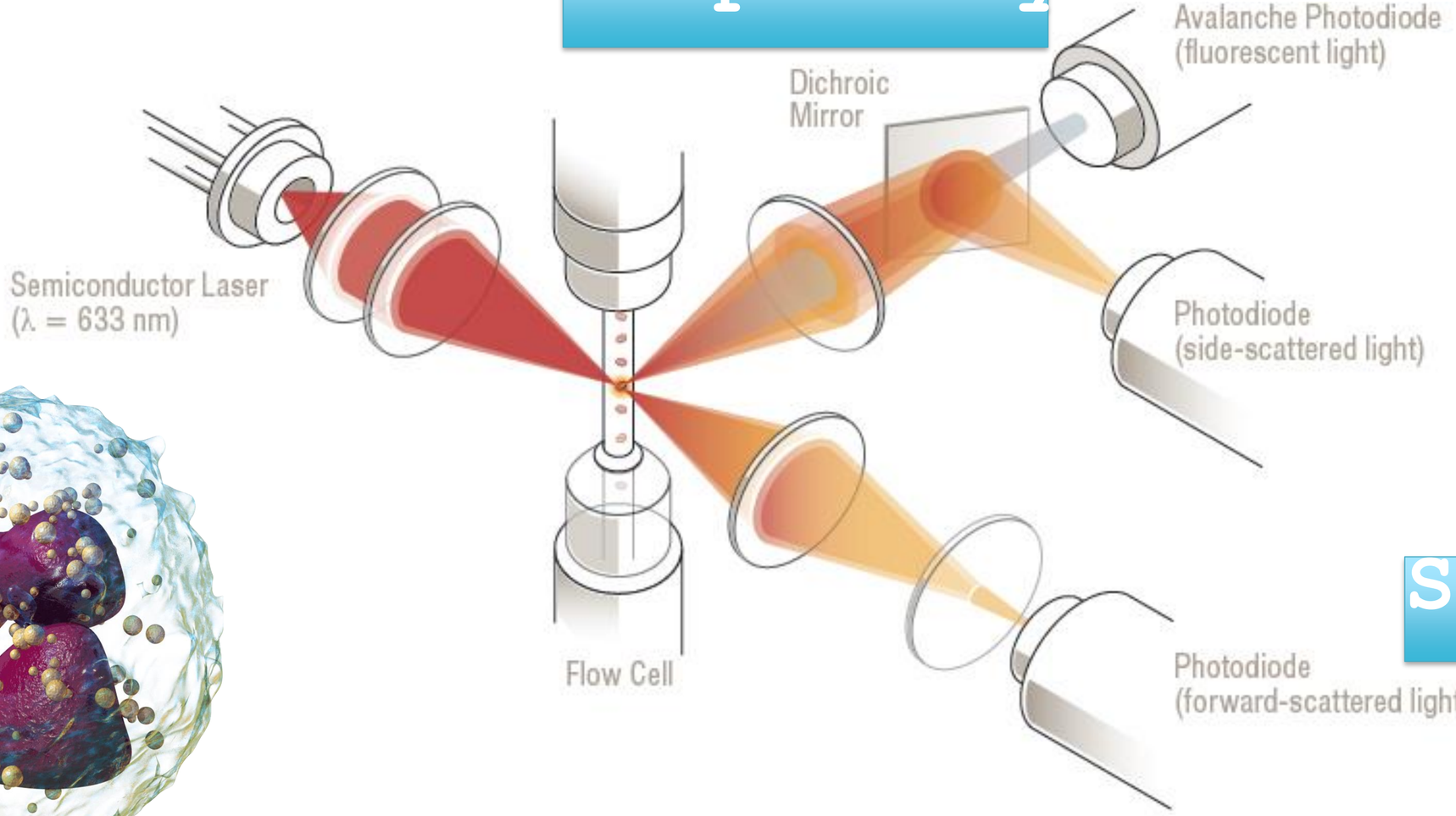
Impedance



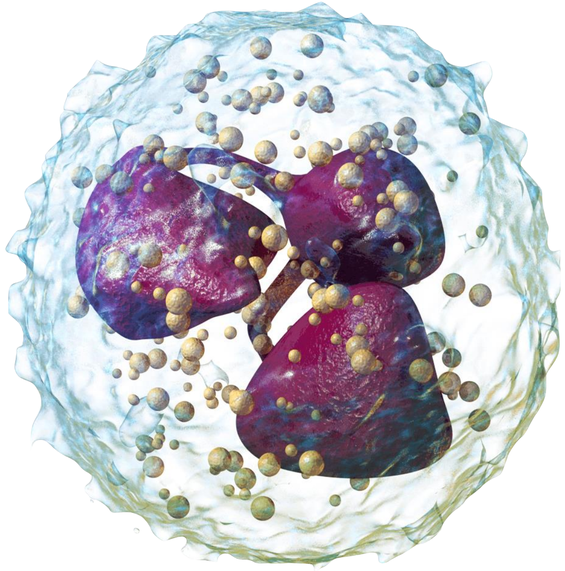


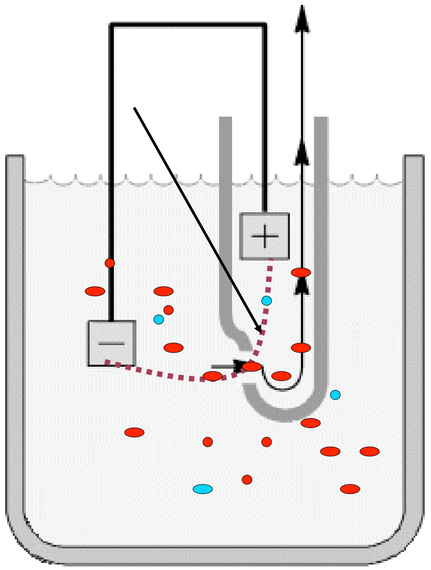
Flow Cytometry-ProCyte Dx

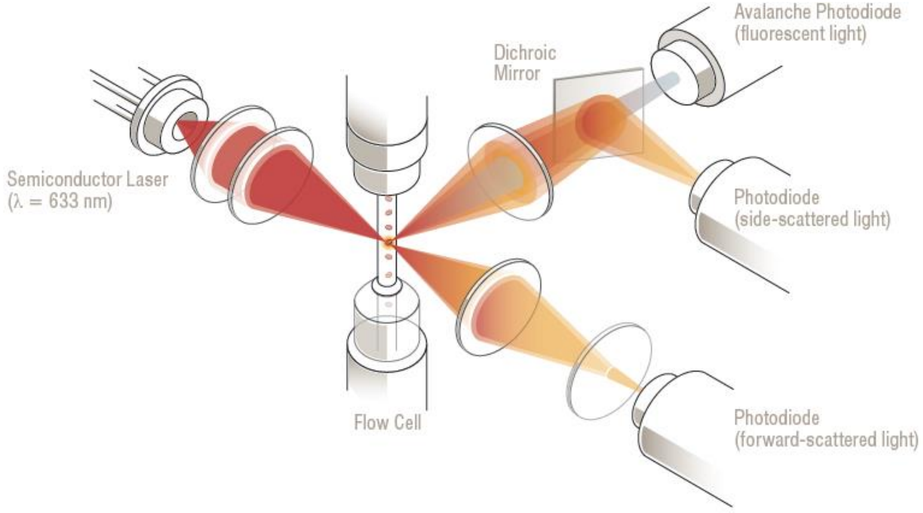
Complexity



Size

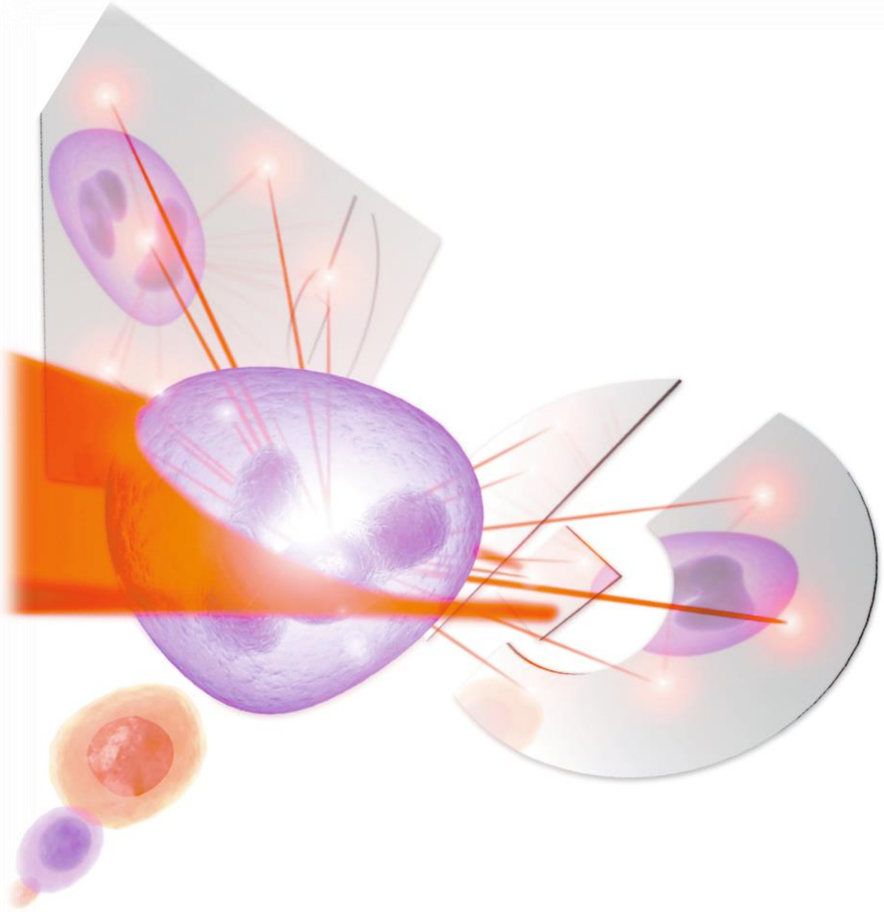






Courtesy Dr. Bill Saxon

Fluorescence

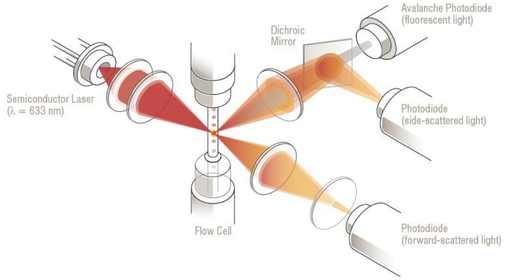


NEUTROPHIL

LYMPHOCYTE

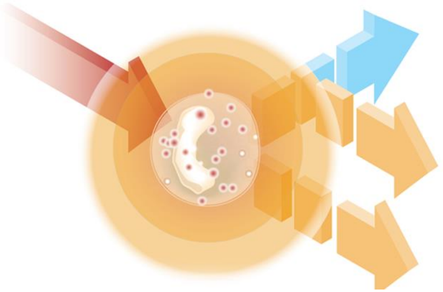
ProCyte Dx

FLOW CYTOMETRY



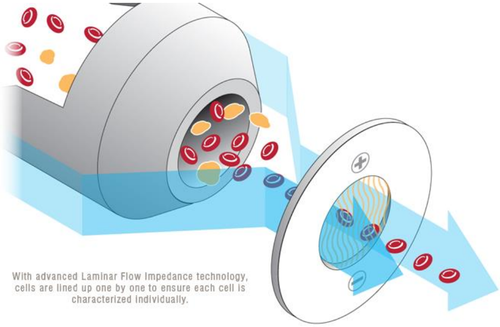
VOLUME/ COMPLEXITY

OPTICAL FLUORESCENCE



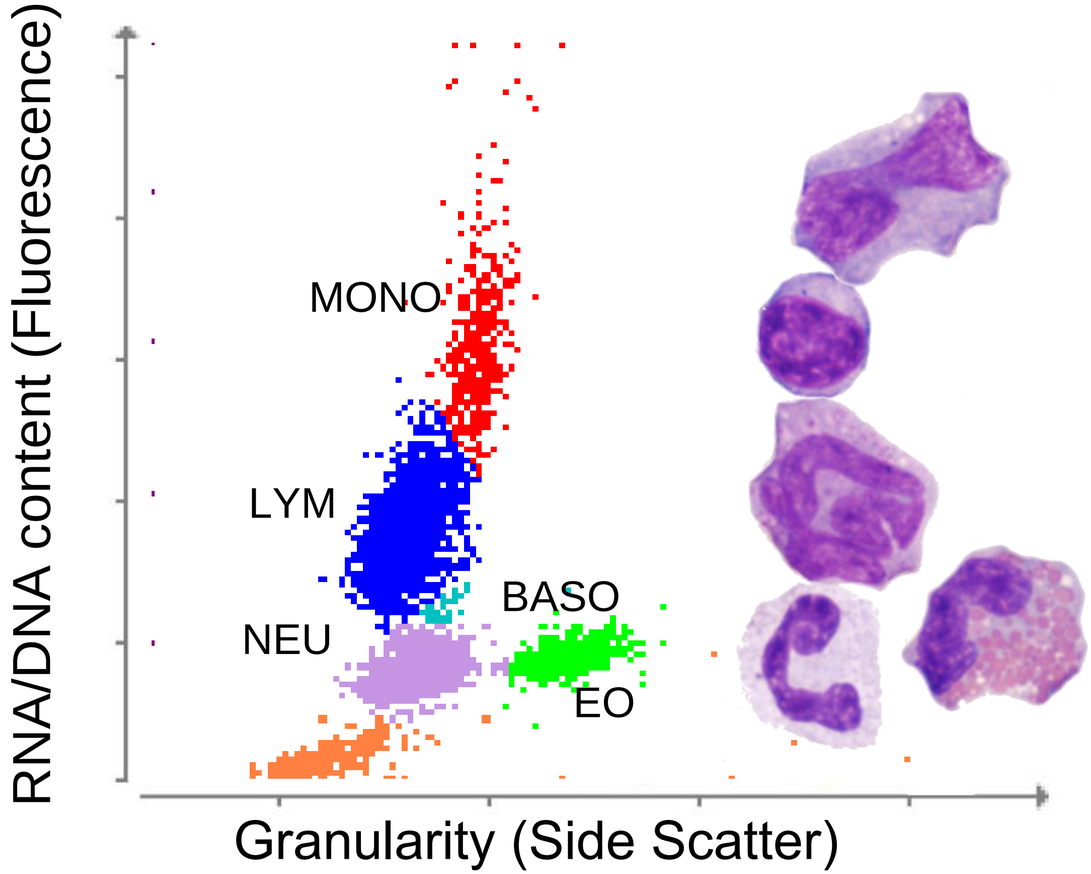
DNA/RNA CONTENT

FLOW IMPEDANCE



NUMBER / VOLUME


IDEXX ProCyte Dx
IDEXX


RNA/DNA content (Fluorescence)
MONO
LYM
BASO
NEU
EO
Granularity (Side Scatter)

Leukocytes

When you do a hand differential you count:

100 cells

A PCDx counts:

>10,000 cells

Hematopoietic Neoplasia

Lymphoma (solid tissue origin)

- Large cell
- Small/Intermediate cell

Leukemias (bone marrow origin)

- Acute (blasts)
- Chronic

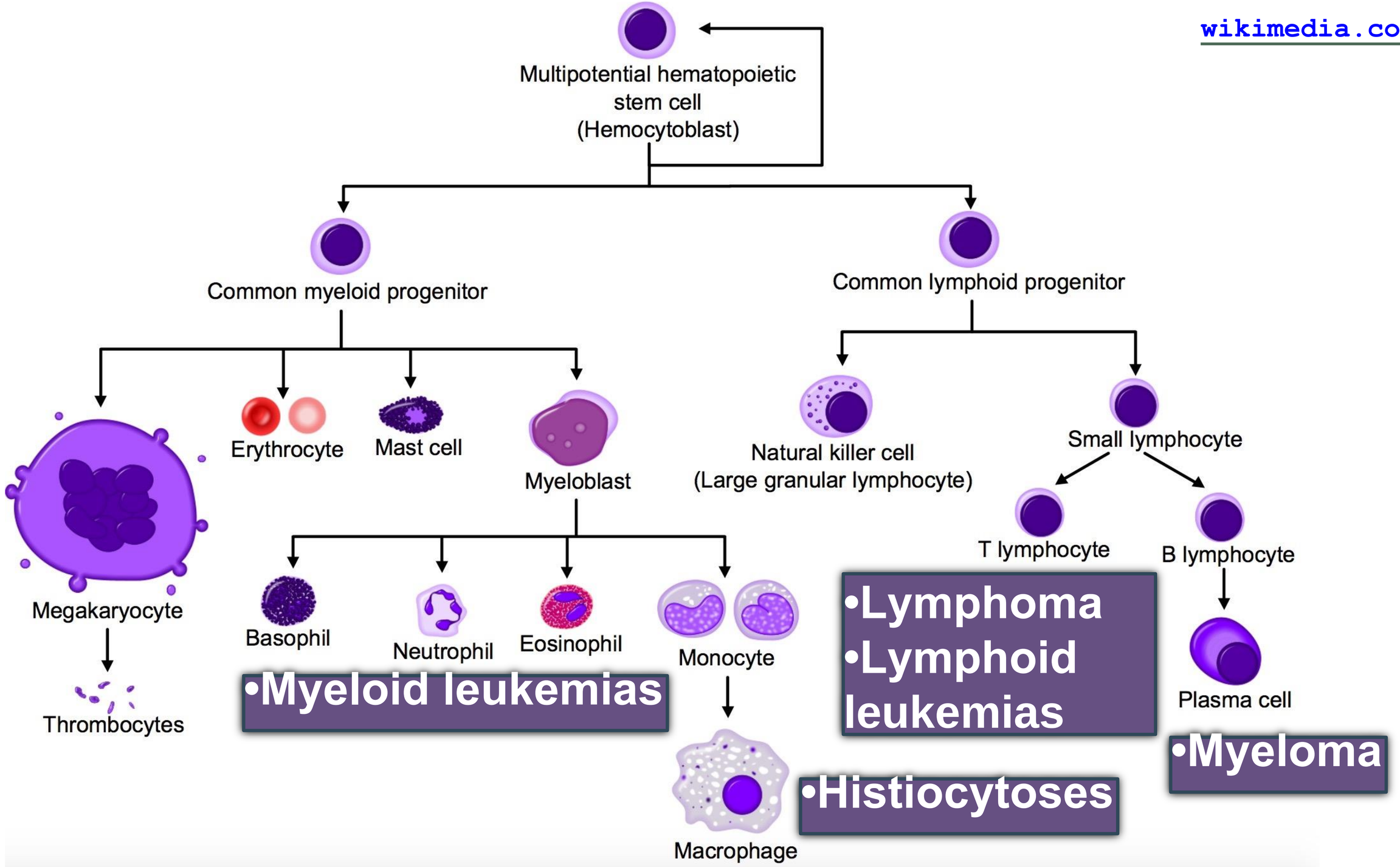
Multiple myeloma

Malignant histiocytosis

Mastocytosis

Etc, etc

wikimedia.com



Hematopoietic Neoplasia

- Most hematopoietic neoplastic cells:
  - Are large
  - Have big nuclei
  - Have dark blue cytoplasm

- Exceptions:
  - Small cell lymphoma
  - Chronic lymphocytic leukemia (CLL)

Hematopoietic Neoplasia

So, if most hematopoietic neoplastic cells:

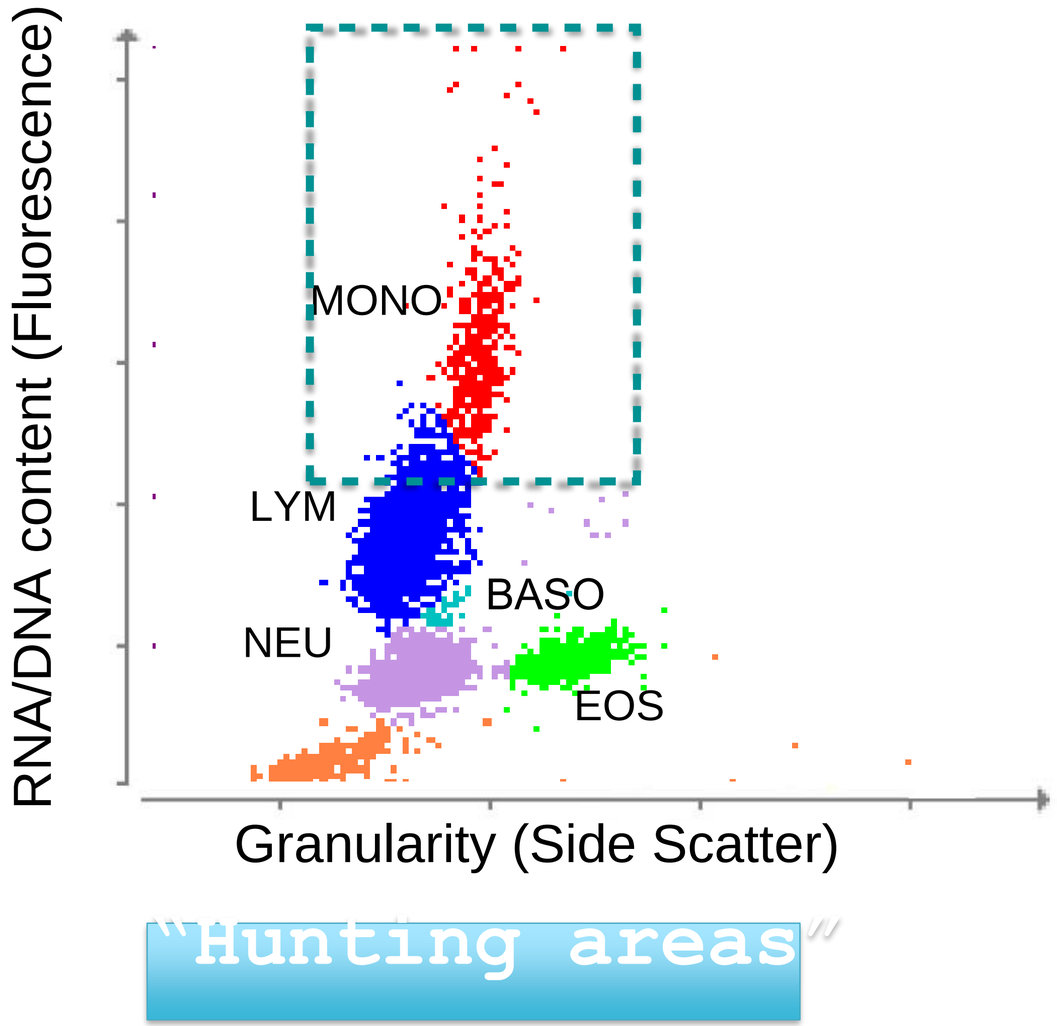
Are large,

Have big nuclei, and

Have dark blue cytoplasm

Where do they “live” in the graphics?

Top floor?, or



"Hunting areas"

Case Discussions

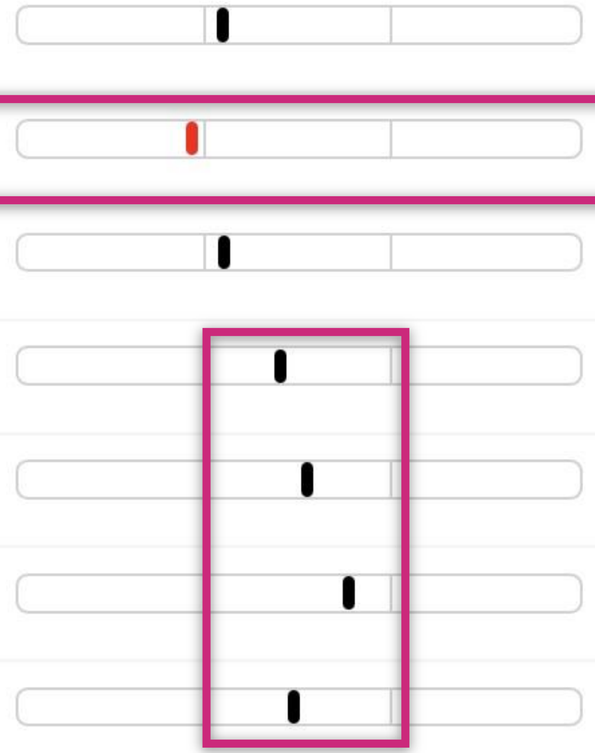
"Simon", 9, MC, DSH

- Referred for hepatic lipidosis

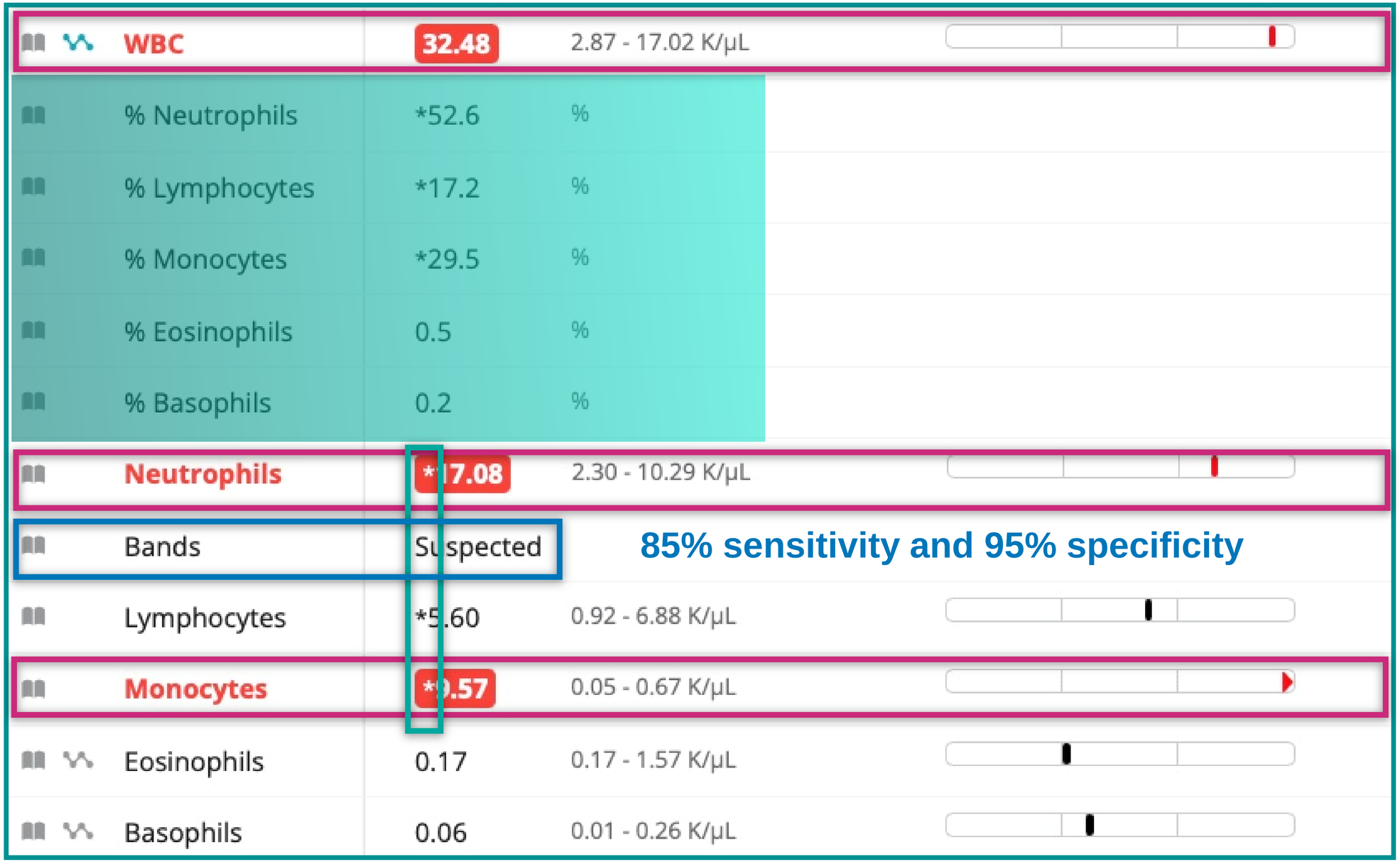
Thanks to Drs. Jason Couto and Kate Sycamore

Simon-CBC-RBCs

RBC	6.88	6.54 - 12.20 x10^12/L
Haematocrit	**0.299**	0.303 - 0.523 L/L
Haemoglobin	103	98 - 162 g/L
MCV	43.5	35.9 - 53.1 fL
MCH	15.0	11.8 - 17.3 pg
MCHC	344	281 - 358 g/L
RDW	21.2	15.0 - 27.0 %
% Reticulocytes	0.2	%
Reticulocytes	14.4	3.0 - 50.0 K/µL
Reticulocyte Haemoglobin	15.6	13.2 - 20.8 pg



Simon-CBC-WBCs



WBC	32.48	2.87 - 17.02 K/µL
% Neutrophils	*52.6	%
% Lymphocytes	*17.2	%
% Monocytes	*29.5	%
% Eosinophils	0.5	%
% Basophils	0.2	%
Neutrophils	*17.08	2.30 - 10.29 K/µL
Bands	Suspected	
Lymphocytes	*5.60	0.92 - 6.88 K/µL
Monocytes	*0.57	0.05 - 0.67 K/µL
Eosinophils	0.17	0.17 - 1.57 K/µL
Basophils	0.06	0.01 - 0.26 K/µL

85% sensitivity and 95% specificity

DDx For Monocytosis

- Chronic neutropenia
- Granulocyte colony-stimulating factor administration
- Increased endogenous or exogenous corticosteroids (especially in dogs)
- Inflammation (eg, infectious vs noninfectious, acute vs chronic)
- Monocytic or monoblastic leukemia (very rare)
- Necrosis and/or tissue destruction (eg, f… …mmune-mediated hemolytic anemia)
- Paraneoplas… …ors (associated with poor prognosis)
  - …nocyte chemotactic protein; possible secretion of granulocyte-…ny-stimulating factor)
  - Osteosarcoma

But, are they really monocytes?

- Recovery from acute bone marrow injury
  - Secondary to administration of a chemotherapeutic agent
  - Secondary to parvovirus infection (rare)

Simon-CBC-Platelets

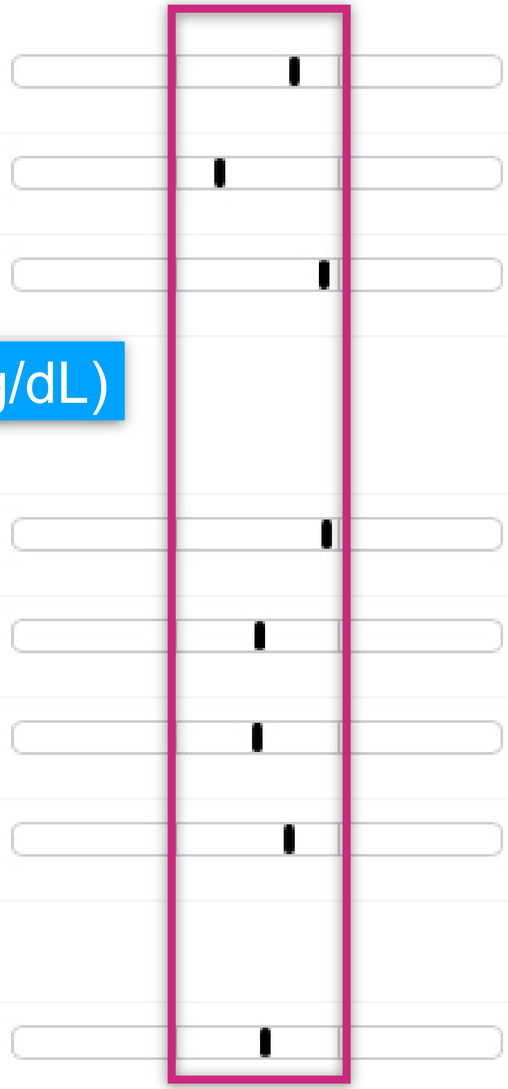
Platelets	50	151 - 600 K/µL
MPV	19.4	11.4 - 21.6 fL
Plateletcrit	0.10	0.17 - 0.86 %

Always evaluate blood smear and/or dot plots for platelet clumping!

Simon-CBC-Chemistry 1

Glucose	139	71 - 159 mg/dL
Creatinine	1.2	0.8 - 2.4 mg/dL
Urea	35	16 - 36 mg/dL
BUN: Creatinine Ratio	30	
Phosphorus	7.4	3.1 - 7.5 mg/dL
Calcium	9.7	7.8 - 11.3 mg/dL
Sodium	158	150 - 165 mmol/L
Potassium	5.2	3.5 - 5.8 mmol/L
Na: K Ratio	30	
Chloride	122	112 - 129 mmol/L

SDMA 40 µg/dL (0-14 µg/dL)



Simon-CBC-Chemistry 2

Total Protein	59	57 - 89 g/L	
Albumin	**22**	23 - 39 g/L	
Globulin	37	28 - 51 g/L	
Albumin: Globulin Ratio	0.6		
ALT	78	12 - 130 U/L	
ALP	**235**	14 - 111 U/L	
GGT	**12**	0 - 4 U/L	
Bilirubin - Total	**34.2**	0 - 15.39 µmol/L	
Cholesterol	4.06	1.68 - 5.82 mmol/L	
Amylase	1,219	500 - 1,500 U/L	
Lipase	203	100 - 1,400 U/L	

Simon-UA

Collection	Cystocentesis	
Colour	Dark Yellow	
Clarity	Clear	
Specific Gravity	1.024	
pH	6.0	
Urine Protein	TR	
Glucose	neg	
Ketones	neg	
Blood / Haemoglobin	250	Ery/µL
Bilirubin	3	mg/dL
Urobilinogen	8	mg/dL

White Blood Cells	1 /HPF
Red Blood Cells	6 /HPF
Bacteria, Cocci	Suspect presence
Bacteria, Rods	None detected
Squamous Epithelial Cells	None detected
Non-Squamous Epithelial Cells	<1 /HPF
Hyaline Casts	None detected
Non-Hyaline Casts	None detected
Calcium Oxalate Dihydrate Crystals	None detected
Struvite Crystals	None detected
Ammonium Biurate Crystals	None detected
Bilirubin Crystals	None detected
Unclassified Crystals	<1 /HPF

Are There Non-Renal Causes Of High SDMA?

- Yep…
- Greyhounds (RI: 0-20µg/dL)
- Lymphoma and lymphoid leukemias

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Veterinary and Comparative Oncology

WILEY

ORIGINAL ARTICLE

The association between symmetric dimethylarginine concentrations and various neoplasms in dogs and cats

Michael J. Coyne | Corie Drake | Donald J. McCrann | David Kincaid

1803 dogs and cats with neoplasia

TABLE 1 Median SDMA and Cr concentrations by tumour type

Tumour type	SDMA µmol/L (range)			Cr µmol/L (range)		
	Case animals	Control animals	*p* value	Case animals	Control animals	*p* value
Canine hemangiosarcoma	0.54 (0.1–1.53)	0.49 (0.2–2.97)	.136	79.6 (17.7–265.2)	79.6 (26.5–875.2)	.105
Canine lipoma	0.44 (0.1–1.24)	0.49 (0.15–2.03)	.004	79.6 (26.5–229.8)	79.6 (26.5–415.5)	.099
Canine lymphoma	3-98.8 µg/dL	0.49 (0.15–2.47)	<.0001	79.6 (17.7–406.6)	79.6 (35.4–919.4)	.897
Canine mammary adenocarcinoma	0.44 (0.1–1.33)	0.49 (0.1–2.82)	.006	61.9 (26.5–327.1)	70.7 (26.5–724.9)	<.0001
Canine mammary carcinoma	0.44 (0.1–2.22)	0.49 (0.05–4.94)	.008	61.9 (26.5–229.8)	79.6 (17.7–574.6)	<.0001
Feline lymphoma	2-98.8 µg/dL	0.54 (0.15–3.46)	<.0001	114.9 (44.2–1007.8)	123.8 (53.0–795.6)	<.0001
Feline visceral mast cell tumour	0.64 (0.25–1.93)	0.54 (0.25–4.35)	.566	132.6 (53.0–716.1)	132.6 (44.2–539.3)	.826

Cancer type	*N*	OR (95% CI)	*p*
Canine lymphoma	307	10.00 (5.98–16.72)	$p < .001$
Feline lymphoma	224	3.04 (1.95–4.73)	$p < .001$
Feline visceral mast cell tumour	55	1.63 (0.67–3.92)	$p = .275$
Canine hemangiosarcoma	230	1.11 (0.66–1.87)	$p = .691$
Canine mammary carcinoma	387	0.49 (0.28–0.84)	$p = .009$
Canine mammary adenocarcinoma	388	0.41 (0.231–0.71)	$p = .001$
Canine lipoma	212	0.39 (0.18–0.85)	$p = .013$

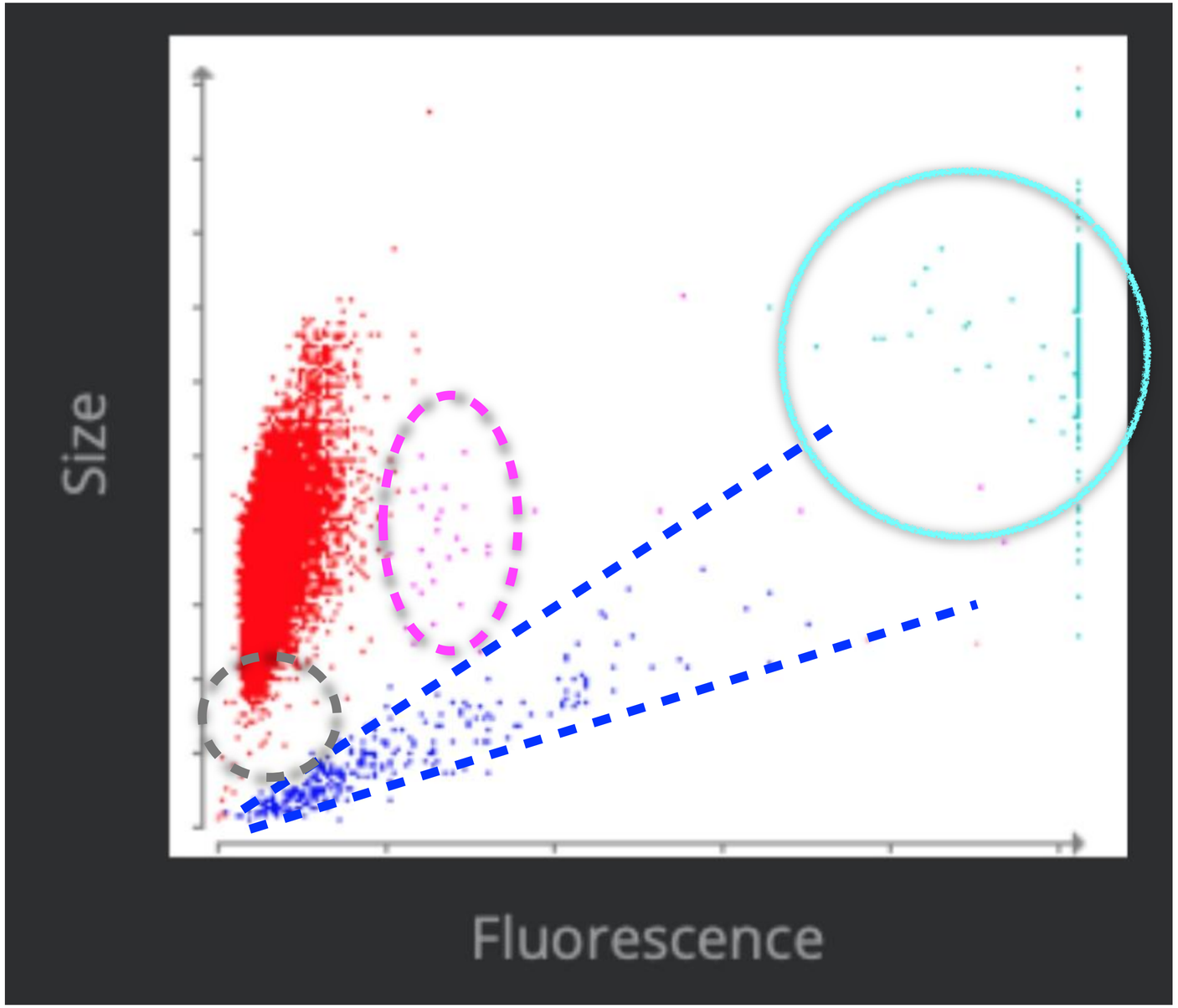
RESEARCH ARTICLE PLOS ONE | https://doi.org/10.1371/journal.pone.0250839 May 14, 2021

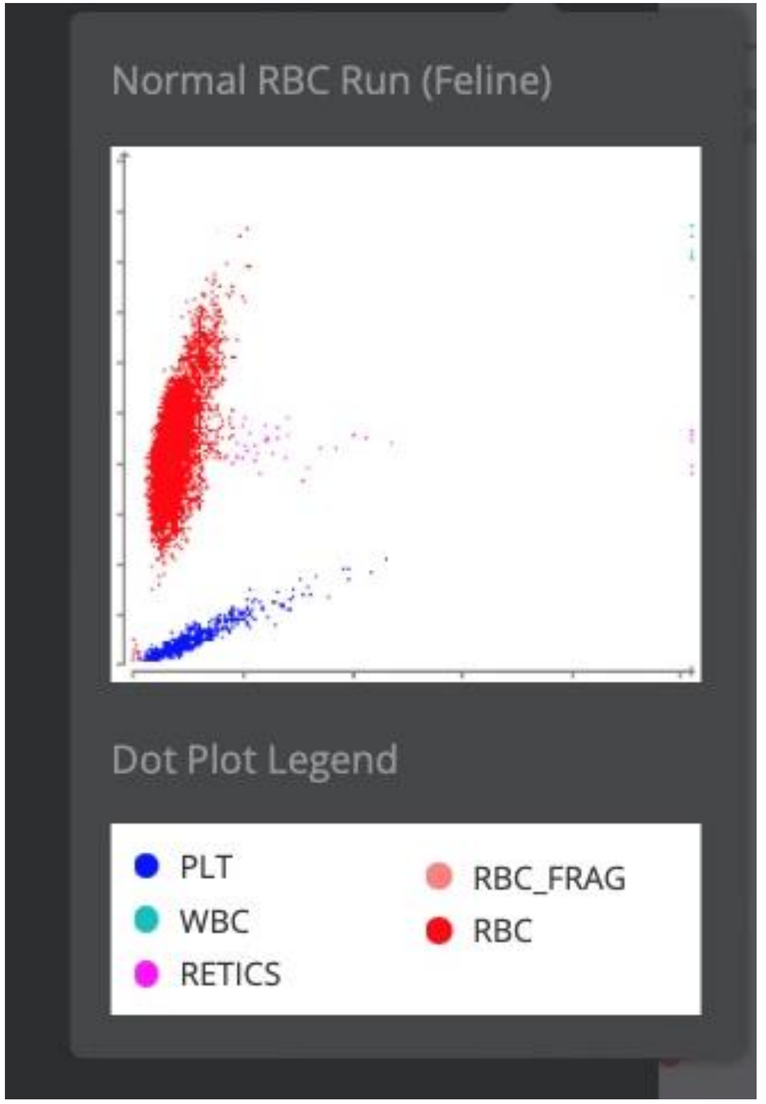
Validation of protein arginine methyltransferase 5 (PRMT5) as a candidate therapeutic target in the spontaneous canine model of non-Hodgkin lymphoma

Shelby L. Sloan[1,2☯], Kyle A. Renaldo[3☯], Mackenzie Long[1,2], Ji-Hyun Chung[2], Lindsay E. Courtney[3], Konstantin Shilo[4], Youssef Youssef[2], Sarah Schlotter[2], Fiona Brown[2], Brett G. Klamer[5], Xiaoli Zhang[5], Ayse S. Yilmaz[5], Hatice G. Ozer[5], Victor E. Valli[6†], Kris Vaddi[7], Peggy Scherle[7], Lapo Alinari[2], William C. Kisseberth[2,3‡*], Robert A. Baiocchi[2‡*]

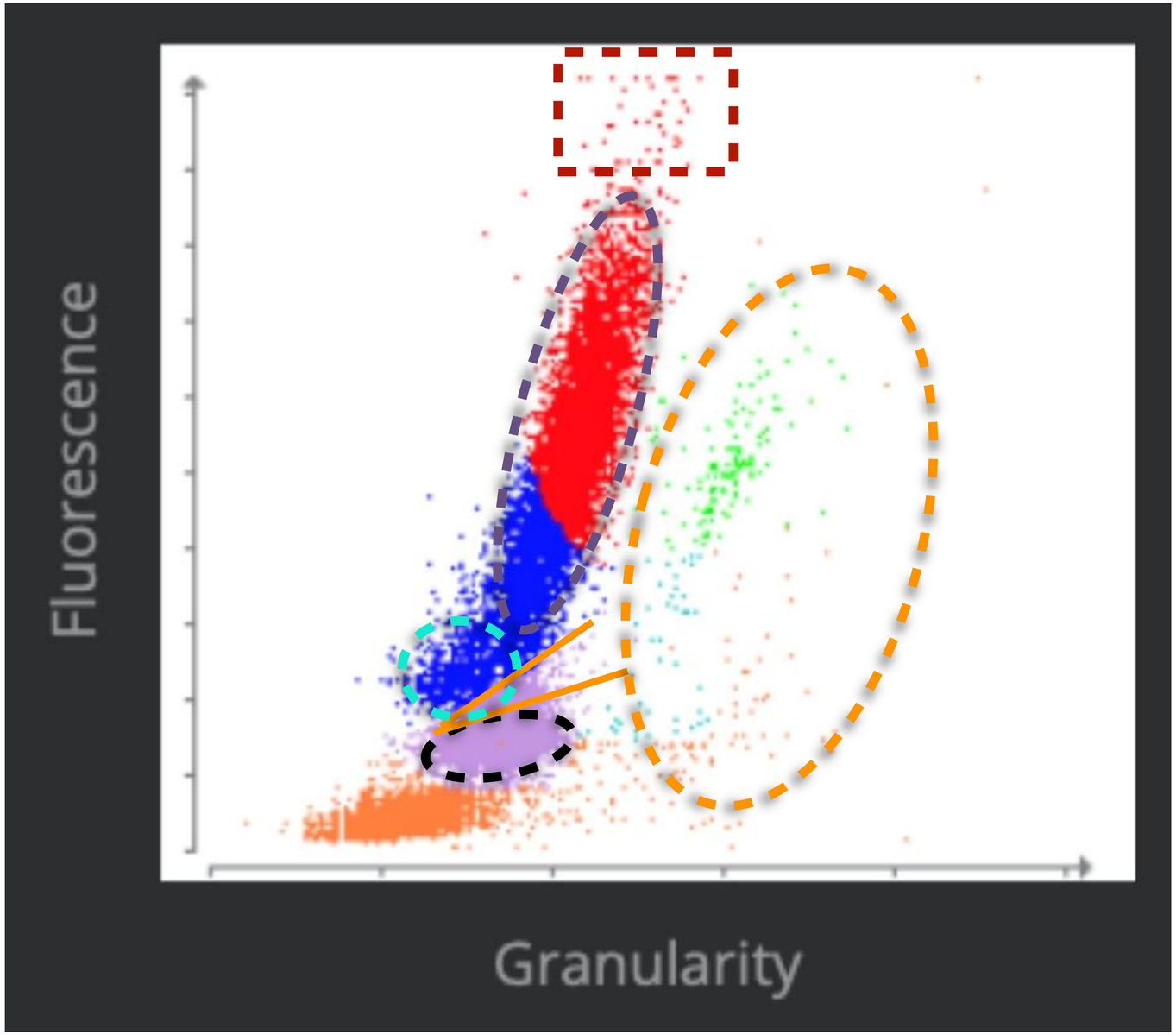
- 42.4% of lymphomas positive for PRMT5
- PRMT5 inhibition → Cell death

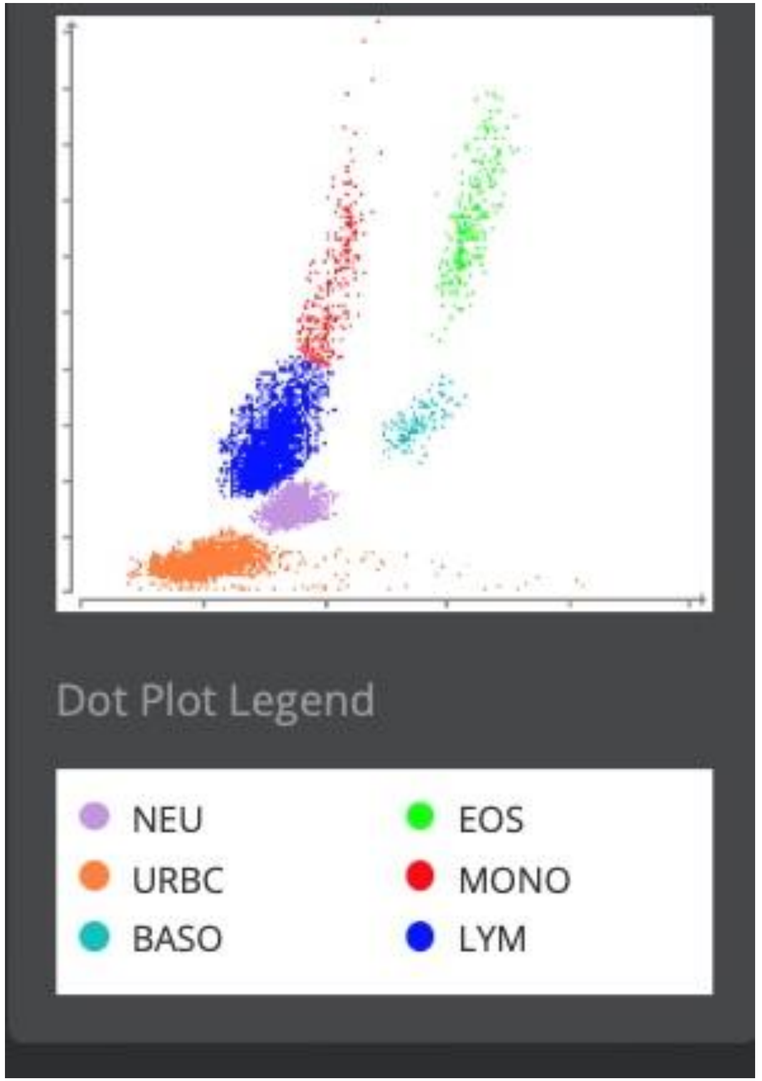
Simon-RBC Dot Plots

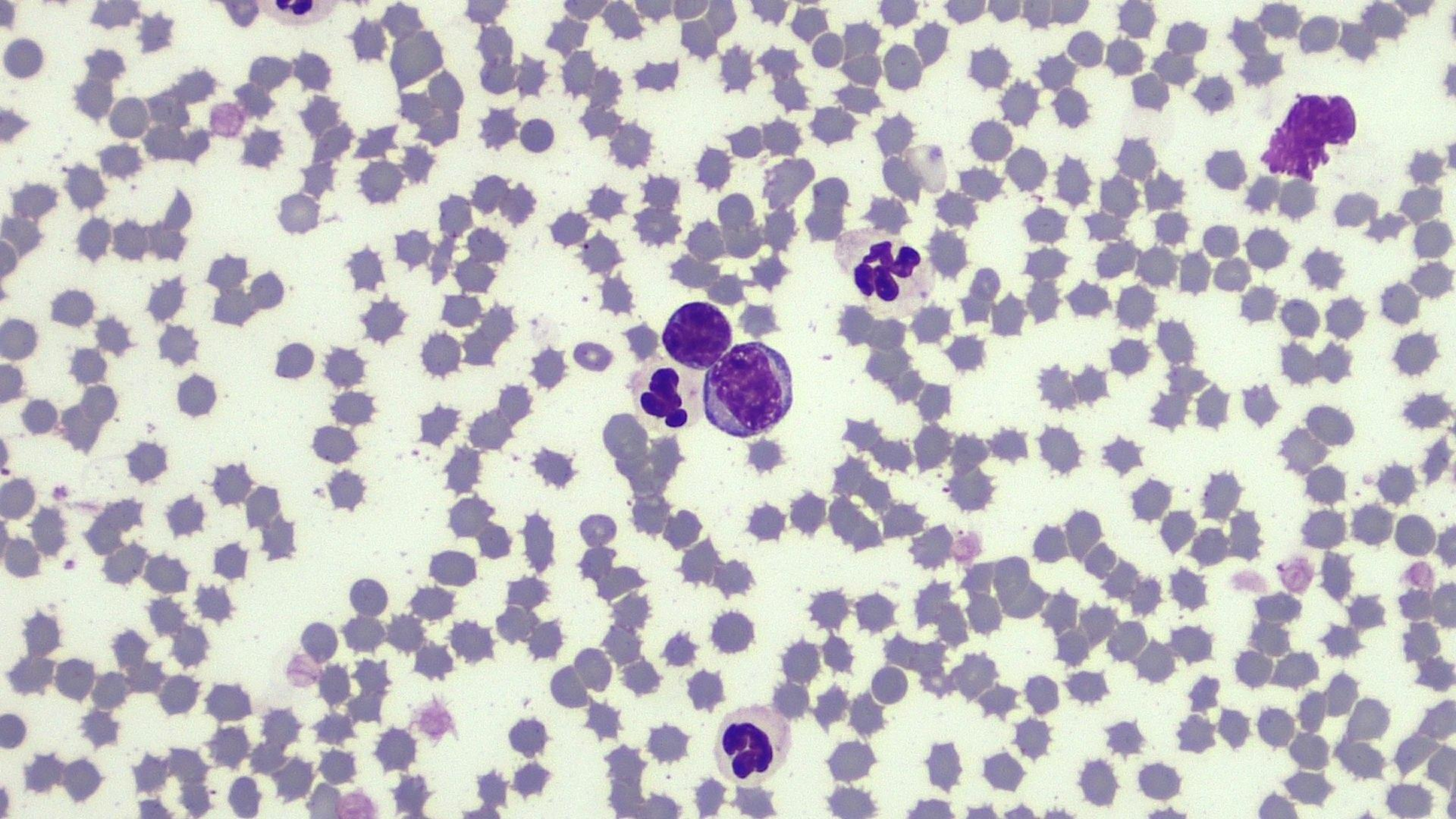


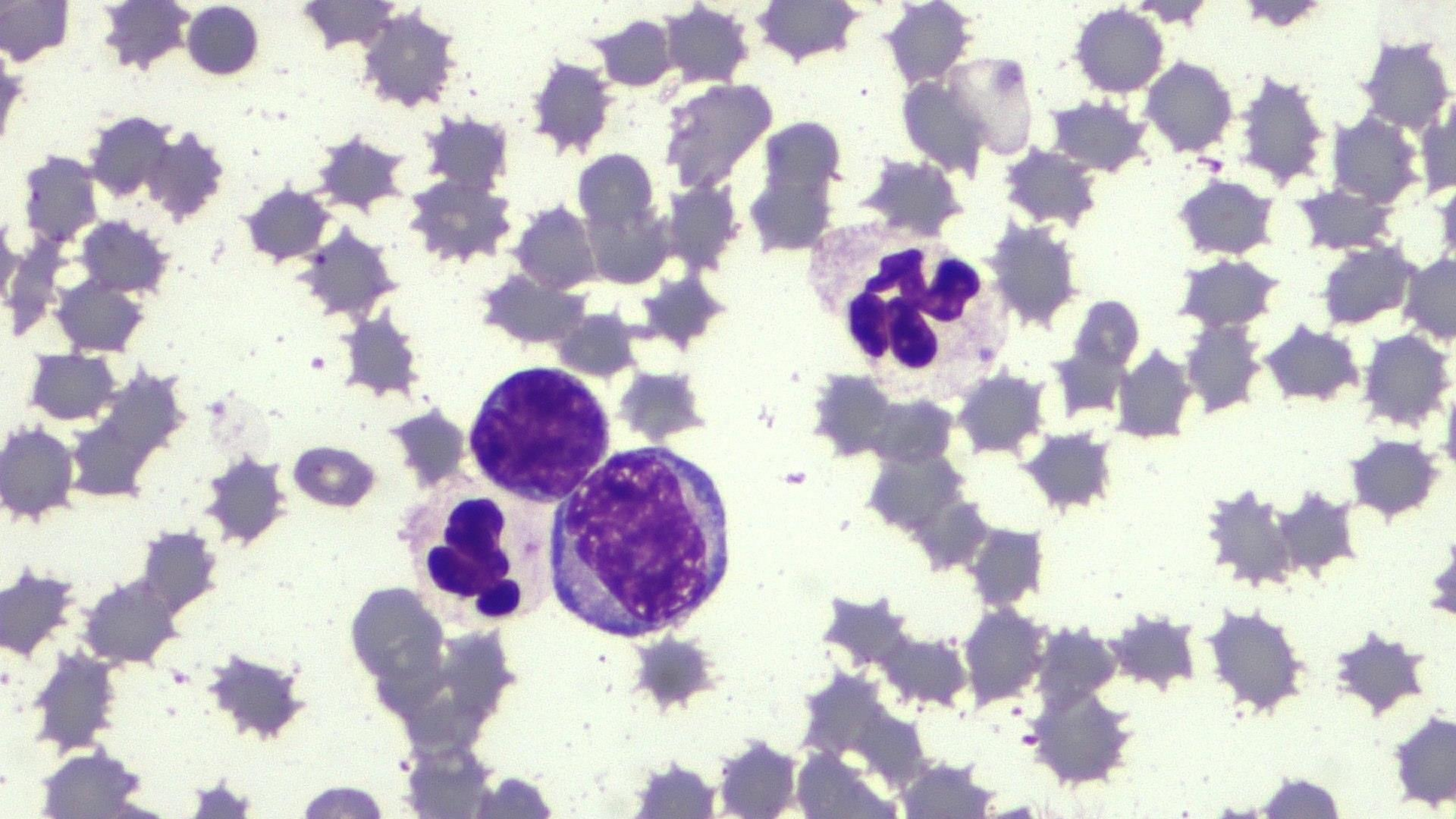


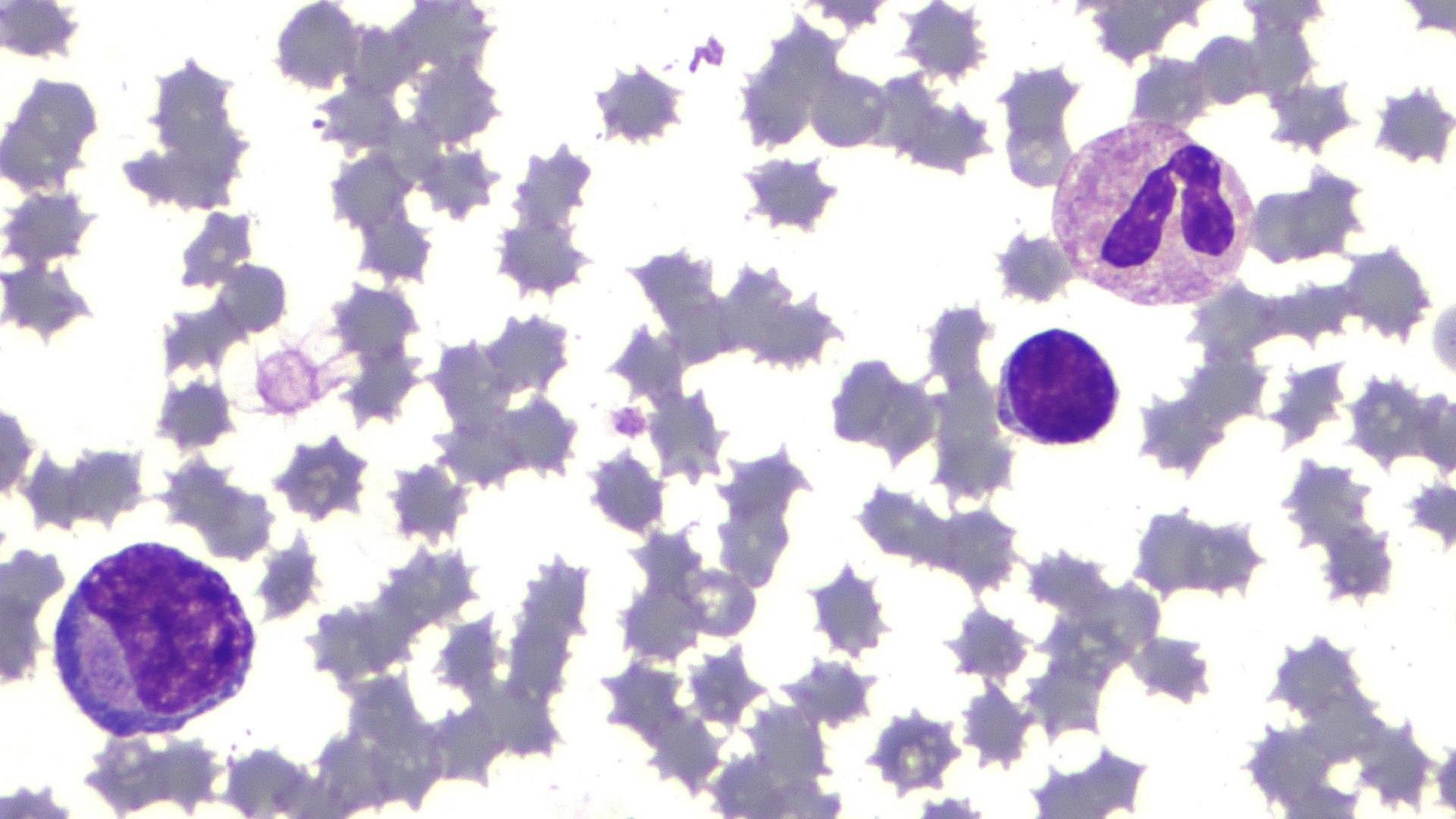
Simon-WBC Dot Plots

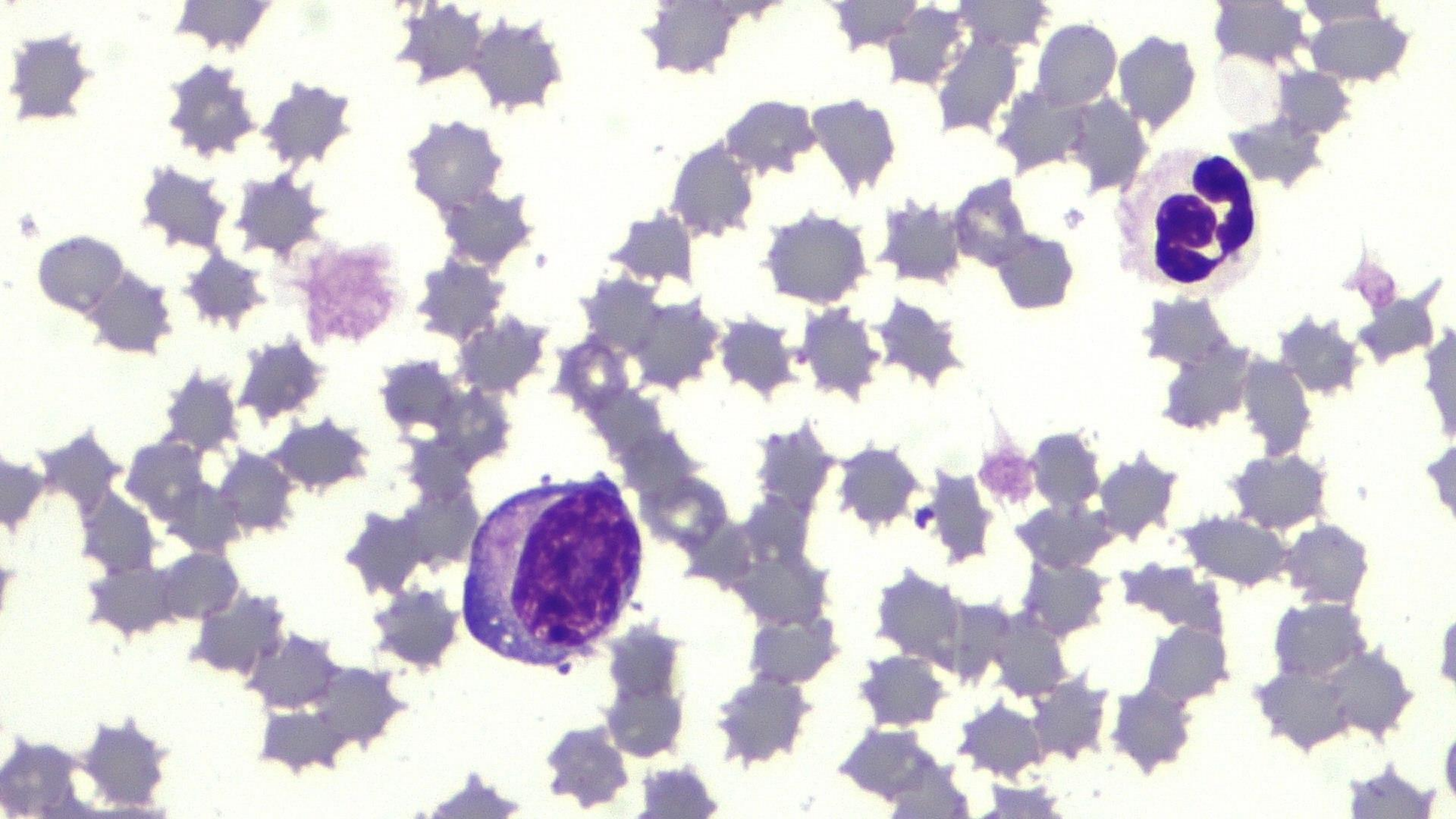




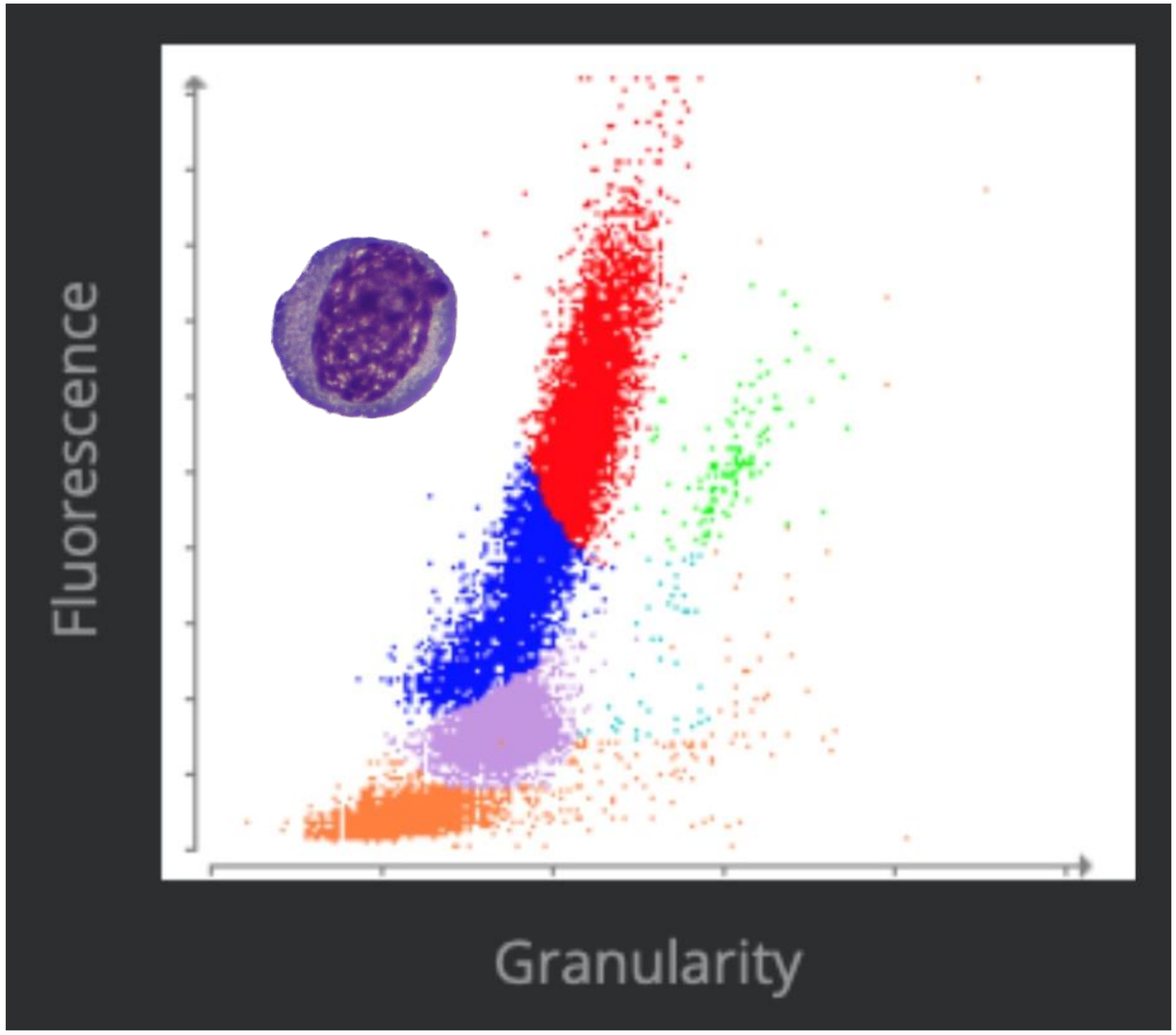


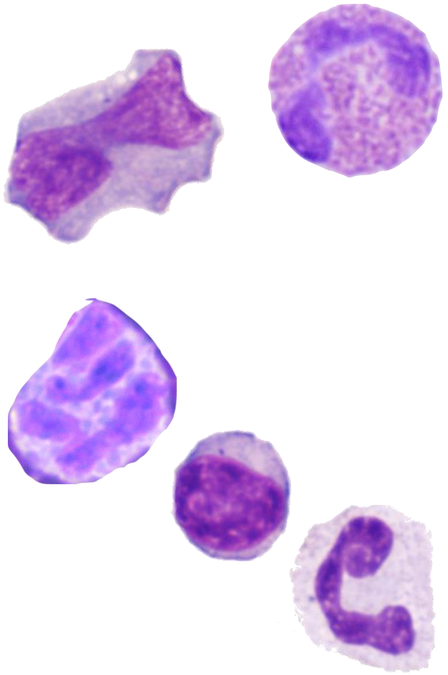






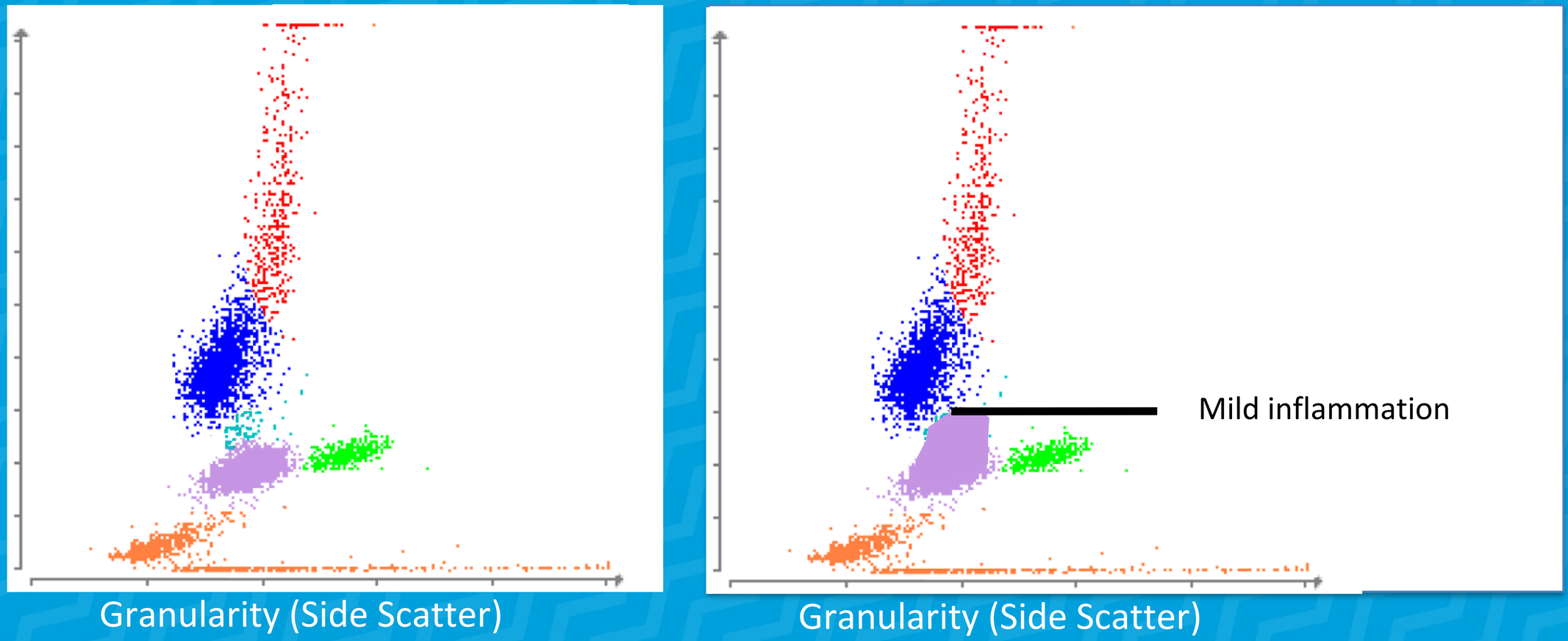
Simon-WBC Dot Plots





ProCyte Dx: White blood cell dot plot

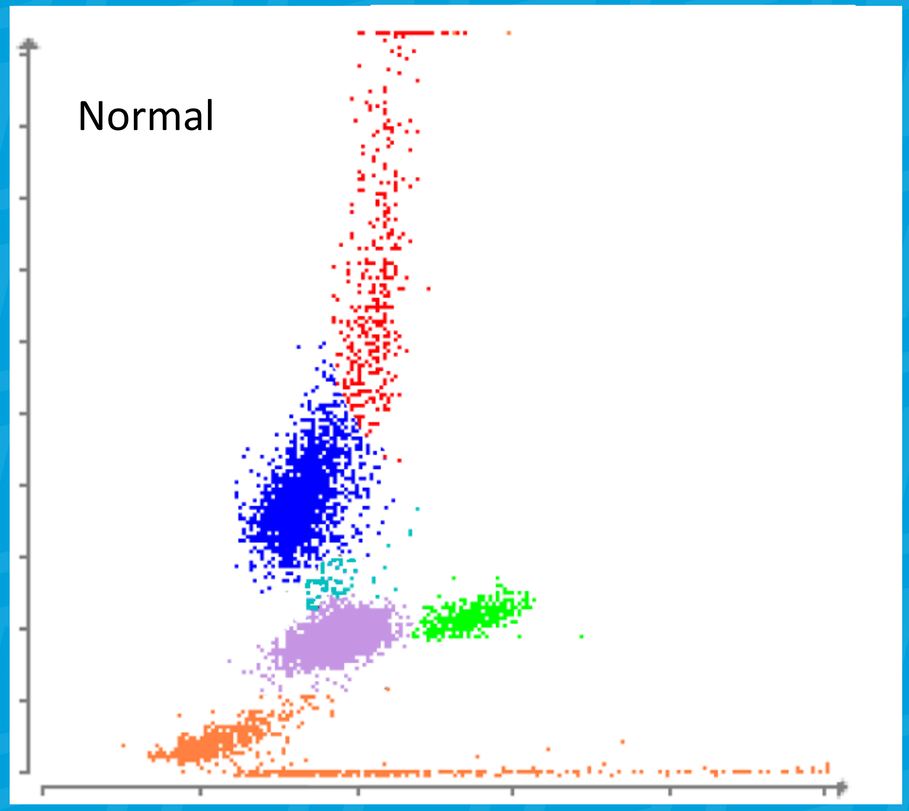
Immature and/or toxic neutrophils

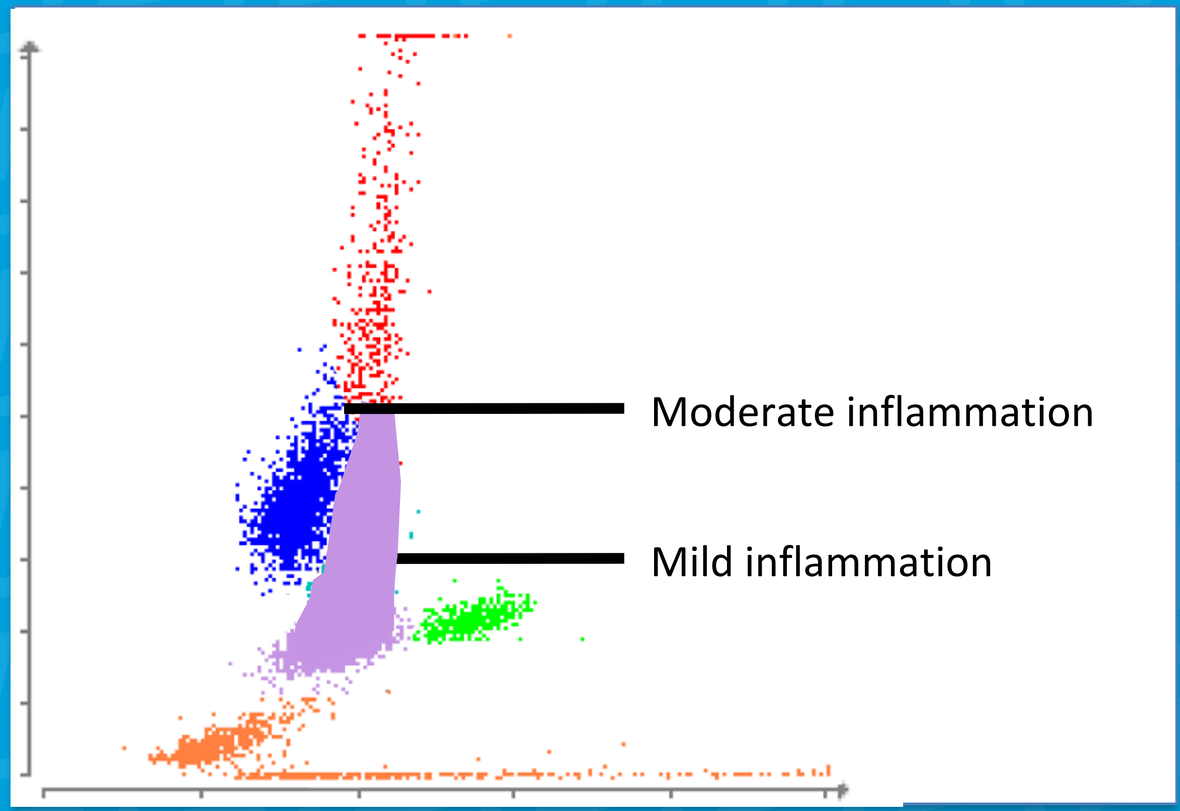


Dr. DeNicola

ProCyte Dx: White blood cell dot plot

Immature and/or toxic neutrophils





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ProCyte Dx: White blood cell dot plot

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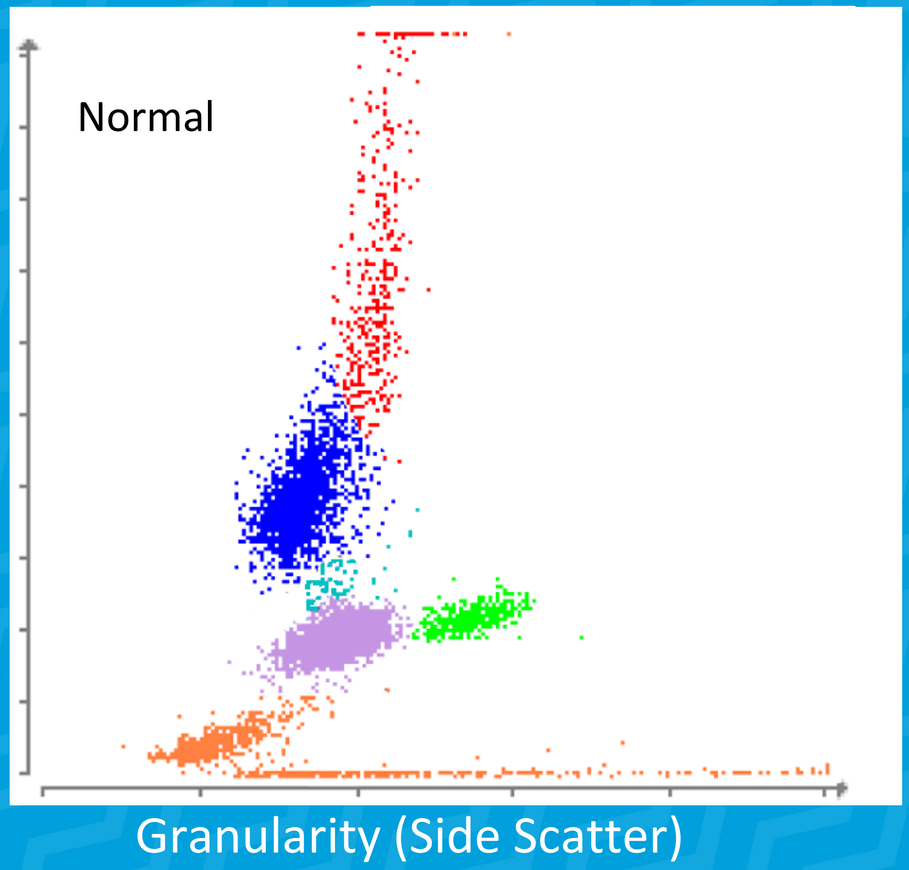
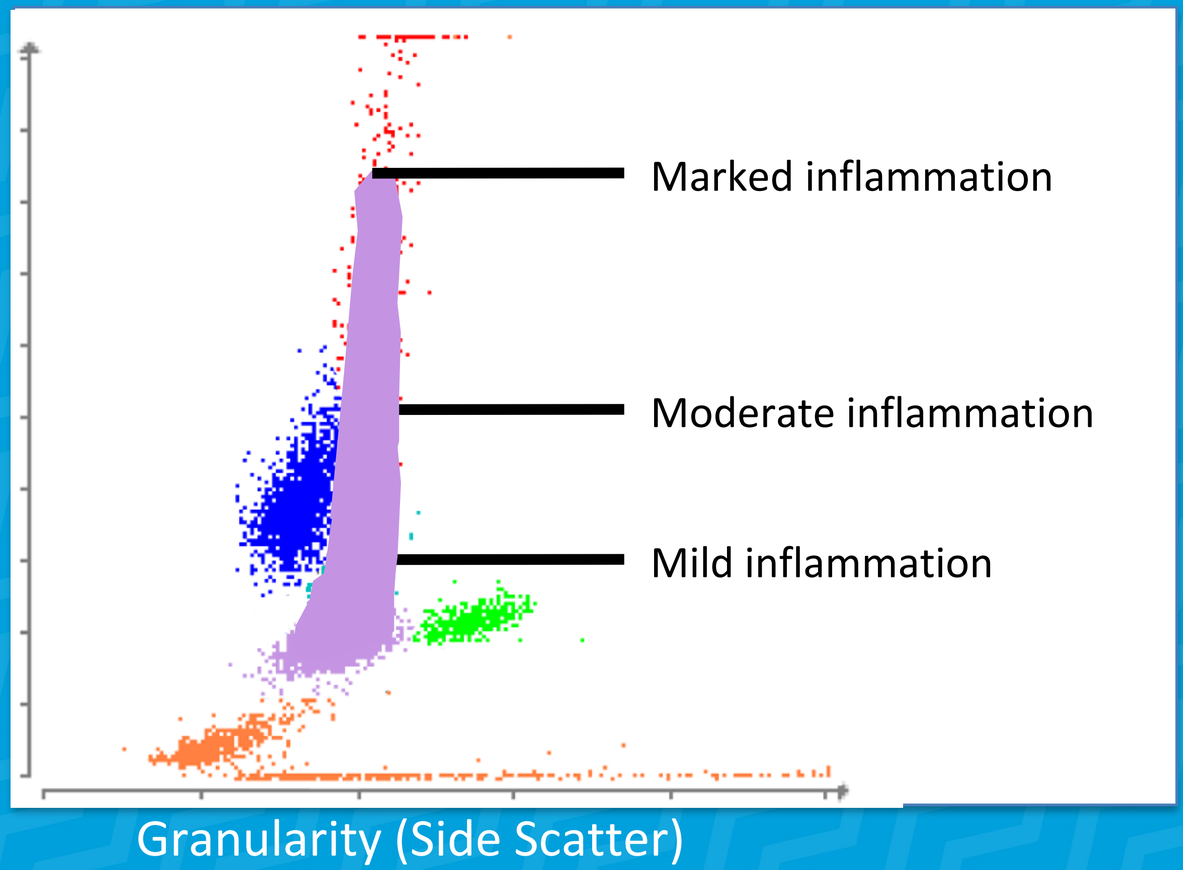
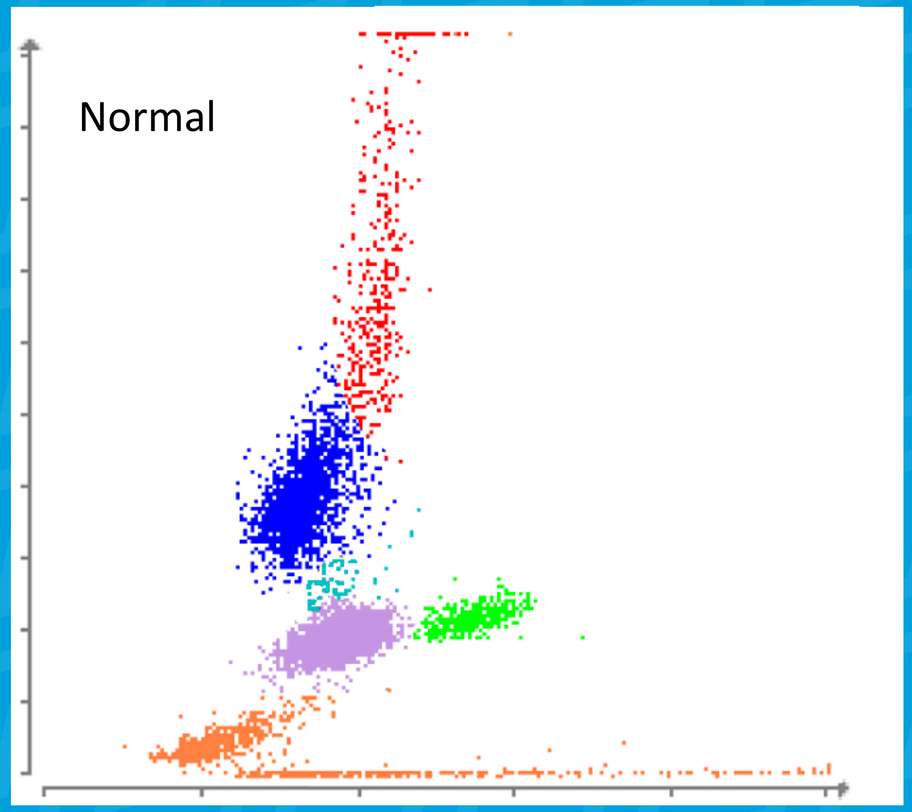
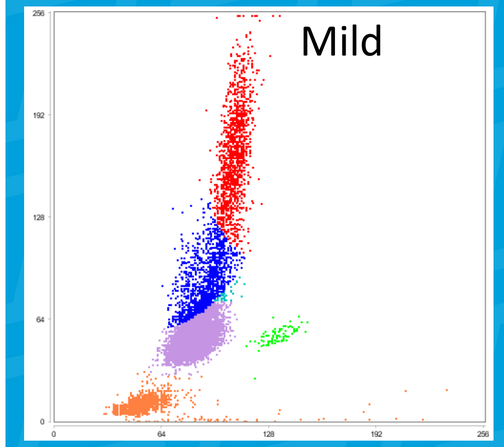


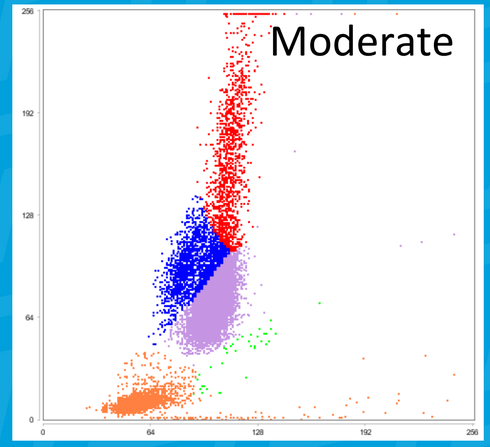


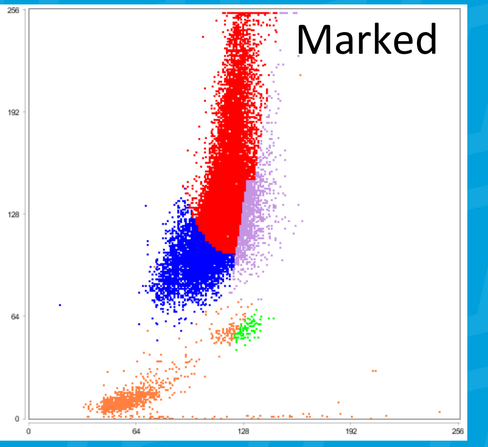
Dr. DeNicola

ProCyte Dx: White blood cell dot plot









Dr. DeNicola

Jack

9-year-old, MC, Beagle

Not feeling well

Generalized lymphadenopathy

Cranial abdominal organomegaly

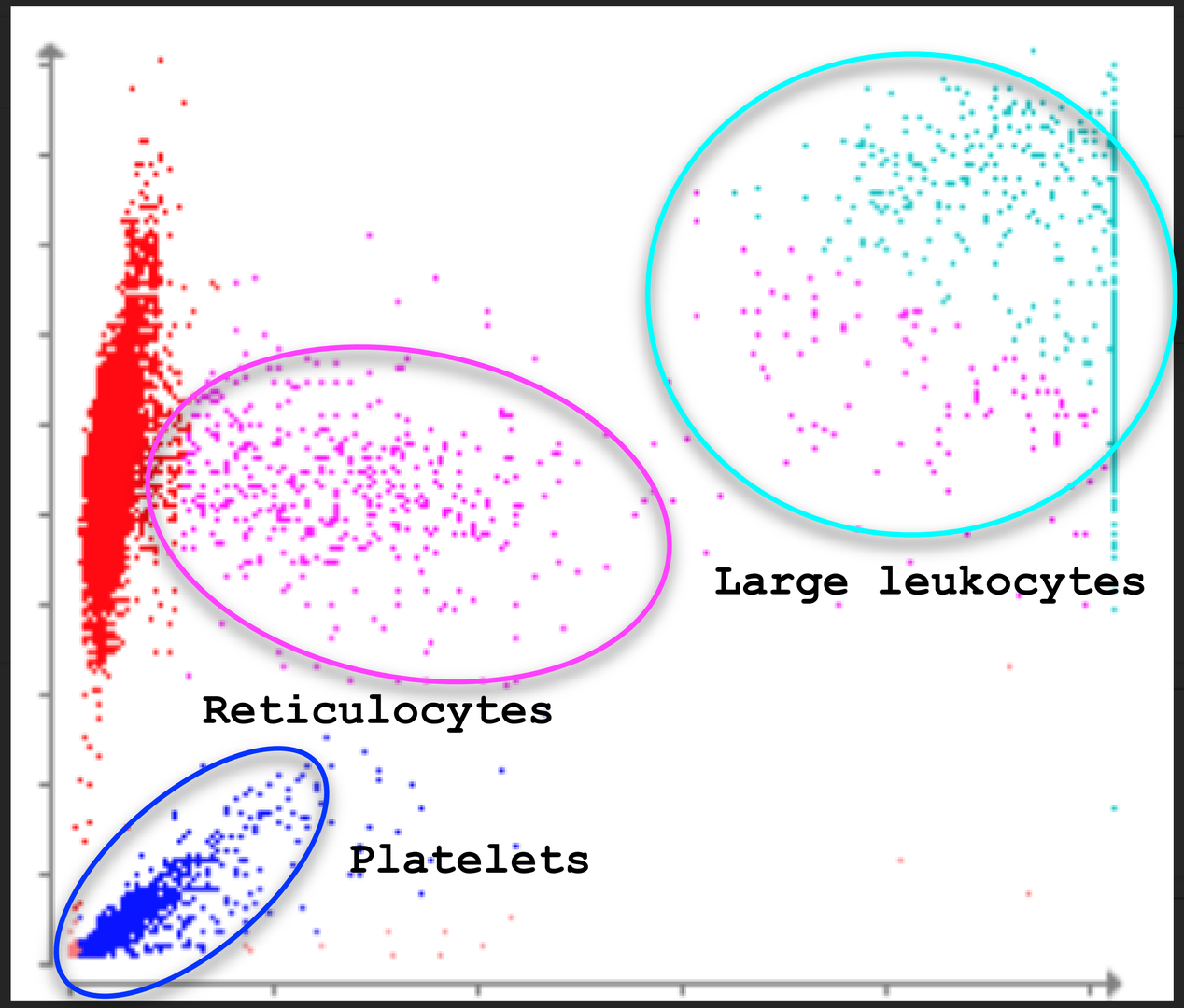
Jack-Chemistry

Does this help wit

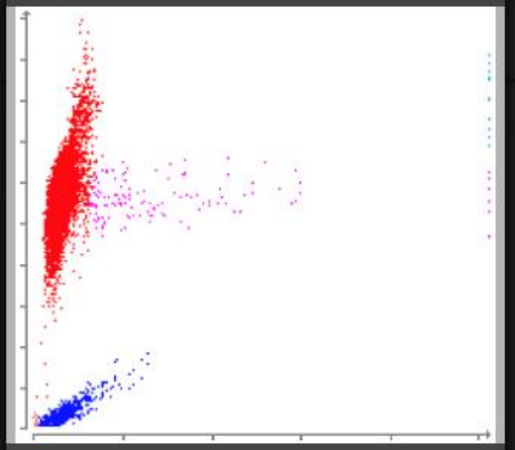
Glucose	6.27	3.89 - 7.94 mmol/L	
IDEXX SDMA a	**64**	0 - 14 µg/dL	
Creatinine	114.92	44.2 - 159.12 µmol/L	
Urea	**16.06**	2.5 - 9.64 mmol/L	
BUN: Creatinine Ratio	35		
Phosphorus	2.07	0.81 - 2.2 mmol/L	
Calcium	2.15	1.97 - 2.99 mmol/L	
Sodium	151	144 - 160 mmol/L	
Potassium	4.4	3.5 - 5.8 mmol/L	
Na: K Ratio	34		
Chloride	115	109 - 122 mmol/L	

JACK [redacted]

RBC Run



NORMAL RBC RUN (Canine)

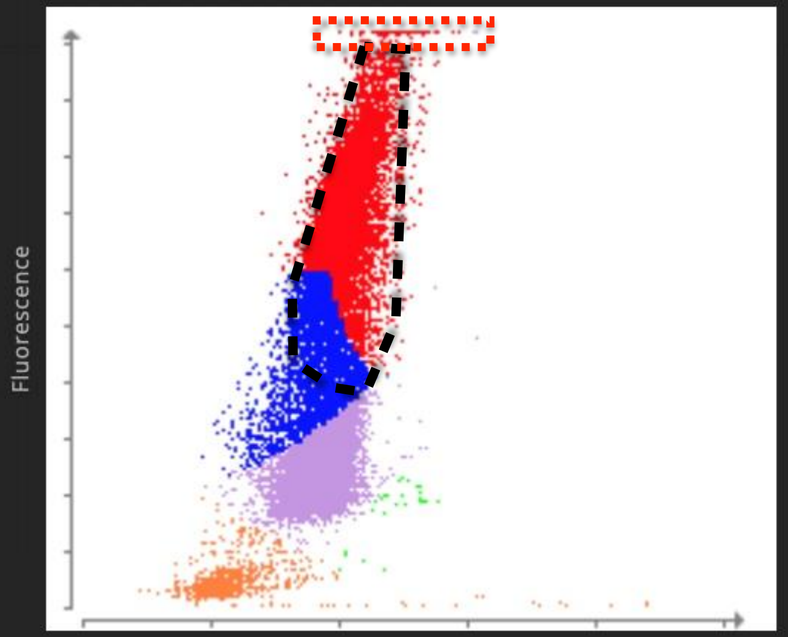


DOT PLOT LEGEND

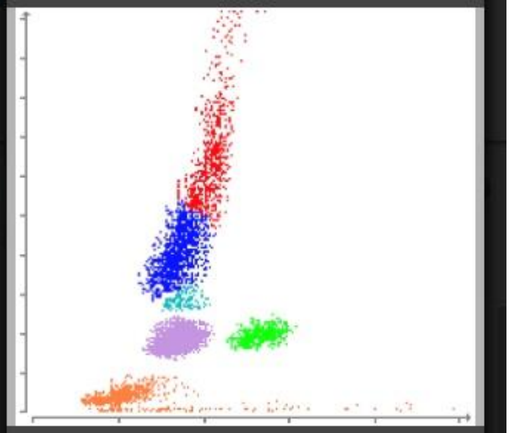
- RBC
- PLT
- RETICS
- RBC_FRAG
- WBC

JACK [redacted]

WBC Run



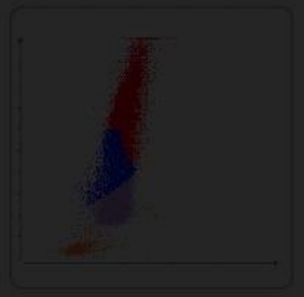
NORMAL WBC RUN (Canine)



DOT PLOT LEGEND

- NEU
- URBC
- BASO
- EOS
- LYM
- MONO

WBC Run



PLT Abnormal Distribution

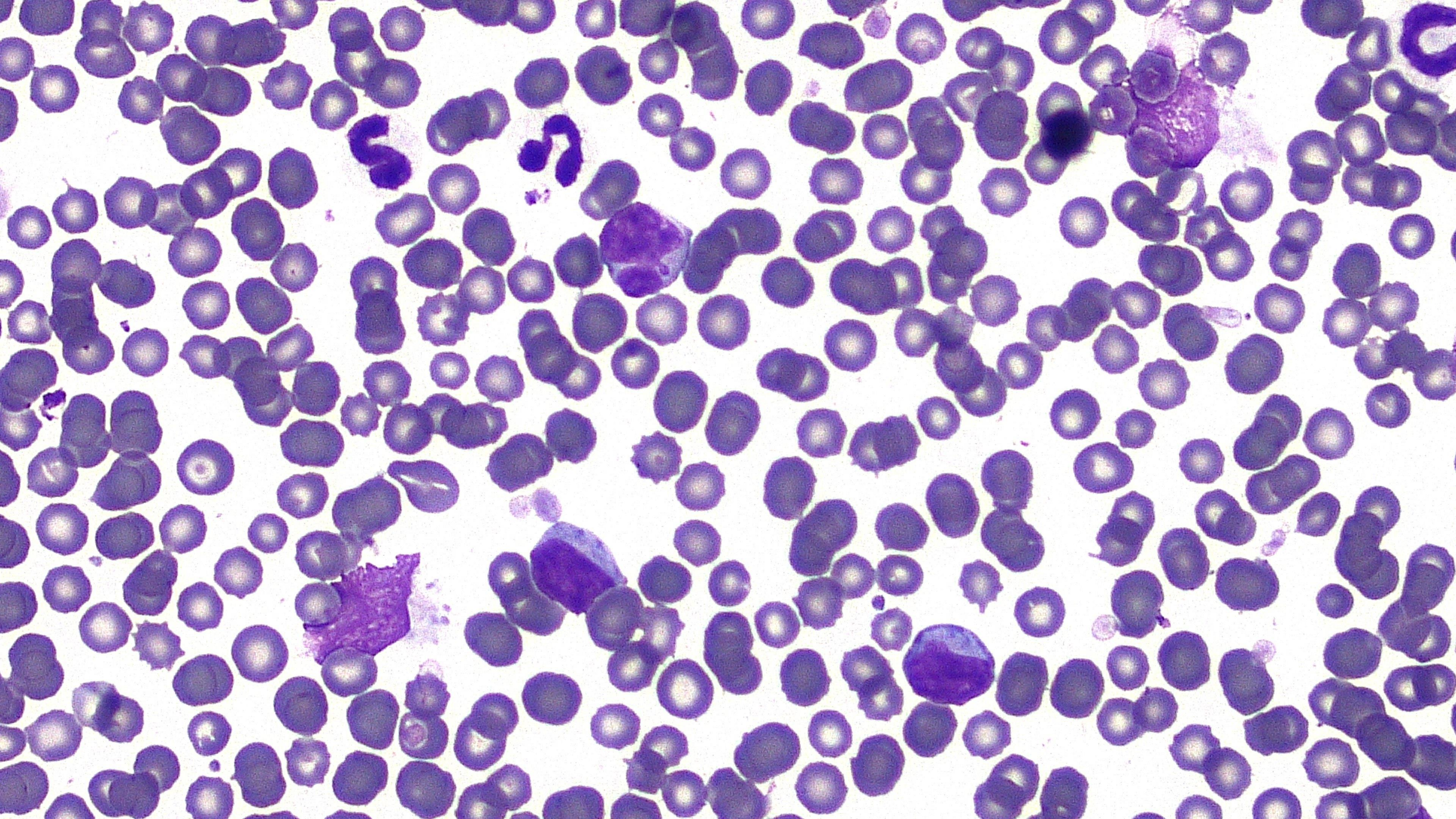
Band neutrophils suspected

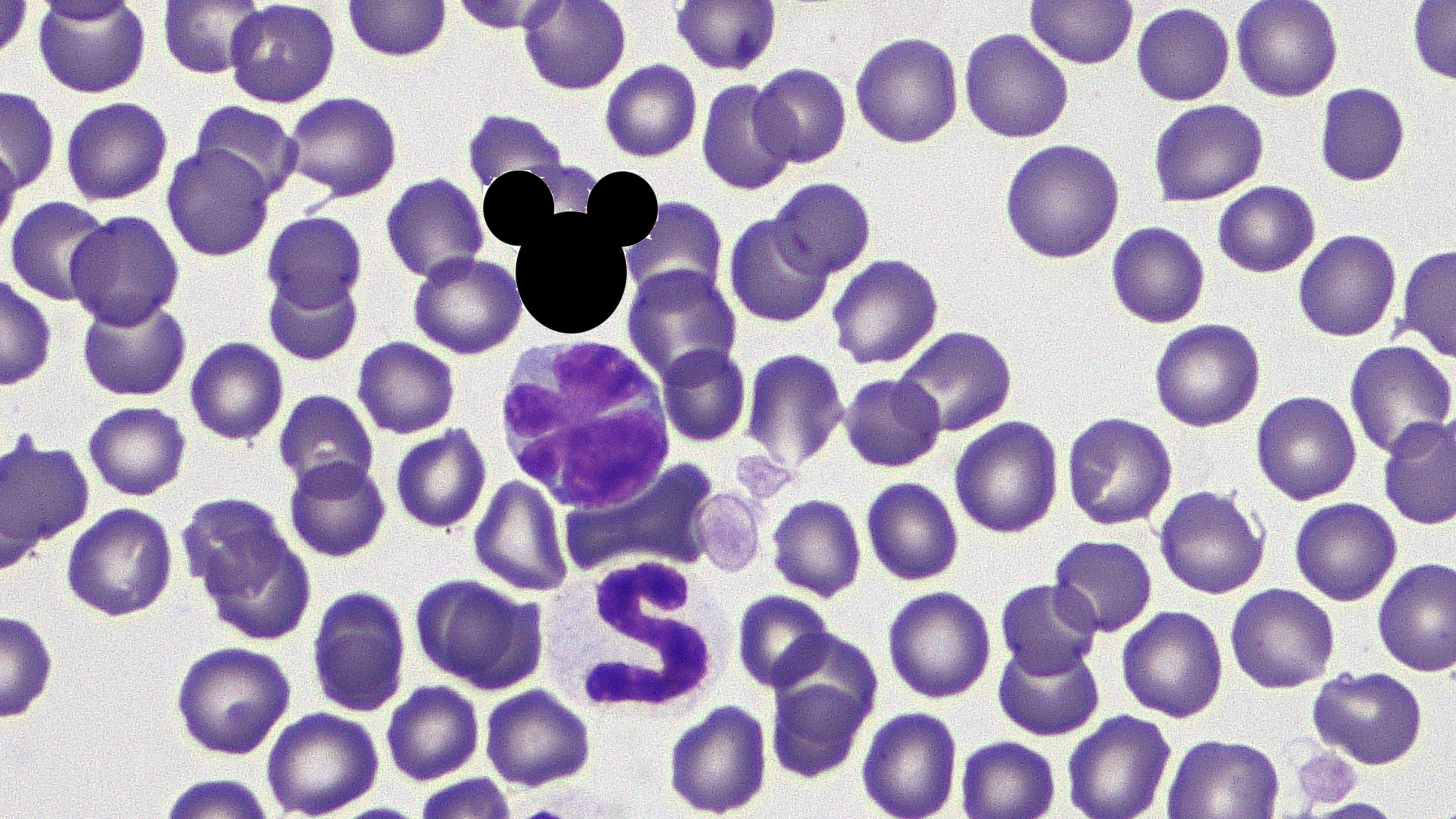
WBC Abnormal Distribution

WBC

IDEXX SDMA

Chemistry	8/1/2019 11:31 AM		9/3/2018 3:46 PM	26/10/2016 3:30 PM
Click to view Differentials				
Glucose	113	70 - 143 mg/dL	129	135





Jack-Numerical Results

RBC	5.85	5.65 - 8.87 M/µL
Haematocrit	**0.36**	0.373 - 0.617 L/L
Haemoglobin	**128**	131 - 205 g/L
MCV	**61.5**	61.6 - 73.5 fL
MCH	21.9	21.2 - 25.9 pg
MCHC	356	320 - 379 g/L
RDW	20.6	13.6 - 21.7 %
% Reticulocytes	2.0	%
Reticulocytes	**117.6**	10.0 - 110.0 K/µL
Reticulocyte Haemoglobin	**21.5**	22.3 - 29.6 pg

WBC	**115.06**	5.05 - 16.76 K/µL
% Neutrophils	34.9	%
% Lymphocytes	20.3	%
% Monocytes	44.7	%
% Eosinophils	0.1	%
% Basophils	0.0	%
Neutrophils	***40.13**	2.95 - 11.64 K/µL
Bands	Suspected	
Lymphocytes	***23.36**	1.05 - 5.1 K/µL
Monocytes	***51.48**	0.16 - 1.12 K/µL
Eosinophils	0.09	0.06 - 1.23 K/µL
Basophils	0	0 - 0.1 K/µL
Platelets	230	148 - 484 K/µL
PDW	--.--	9.1 - 19.4 fL
MPV	--.--	8.7 - 13.2 fL
Plateletcrit	--.--	0.14 - 0.46 %

Jack

Diagnosis:

large cell (T-cell?) multicentric lymphoma

“Charlie”

7, MC, DSH

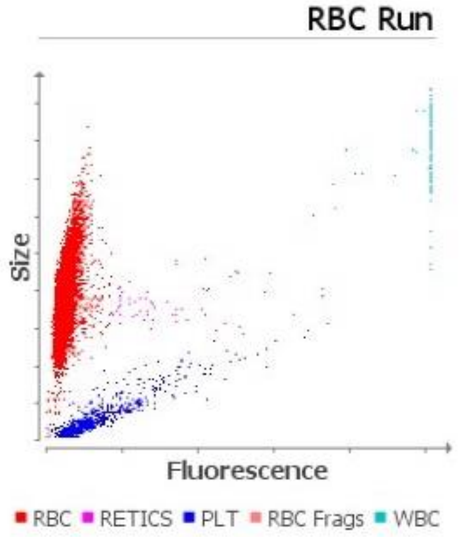
Presented on 8/20/15 for chronic upper airway infection.

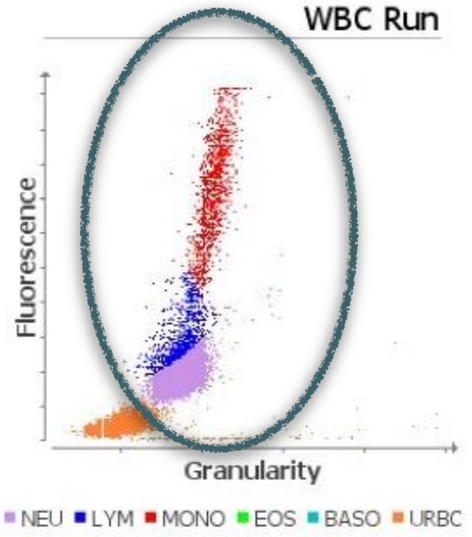
Culture positive for Pseudomonas spp.

Failed topical and systemic antibiotic therapy

Test	Results	Reference Interval	LOW / NORMAL / HIGH
ProCyte Dx (August 20, 2015 8:07 AM)			
RBC	* 9.74 M/µL	6.54 - 12.20	
HCT	* 39.6 %	30.3 - 52.3	
HGB	13.3 g/dL	9.8 - 16.2	
MCV	* 40.7 fL	35.9 - 53.1	
MCH	* 13.7 pg	11.8 - 17.3	
MCHC	* 33.6 g/dL	28.1 - 35.8	
RDW	* 32.0 %	15.0 - 27.0	HIGH
%RETIC	0.2 %		
RETIC	* 23.4 K/µL	3.0 - 50.0	
WBC	36.27 K/µL	2.87 - 17.02	HIGH
%NEU	90.7 %		
%LYM	4.4 %		
%MONO	4.9 %		
%EOS	0.0 %		
%BASO	0.0 %		
NEU	32.88 K/µL	1.48 - 10.29	HIGH
LYM	1.60 K/µL	0.92 - 6.88	
MONO	1.77 K/µL	0.05 - 0.67	HIGH
EOS	0.01 K/µL	0.17 - 1.57	LOW
BASO	0.01 K/µL	0.01 - 0.26	
PLT	326 K/µL	151 - 600	

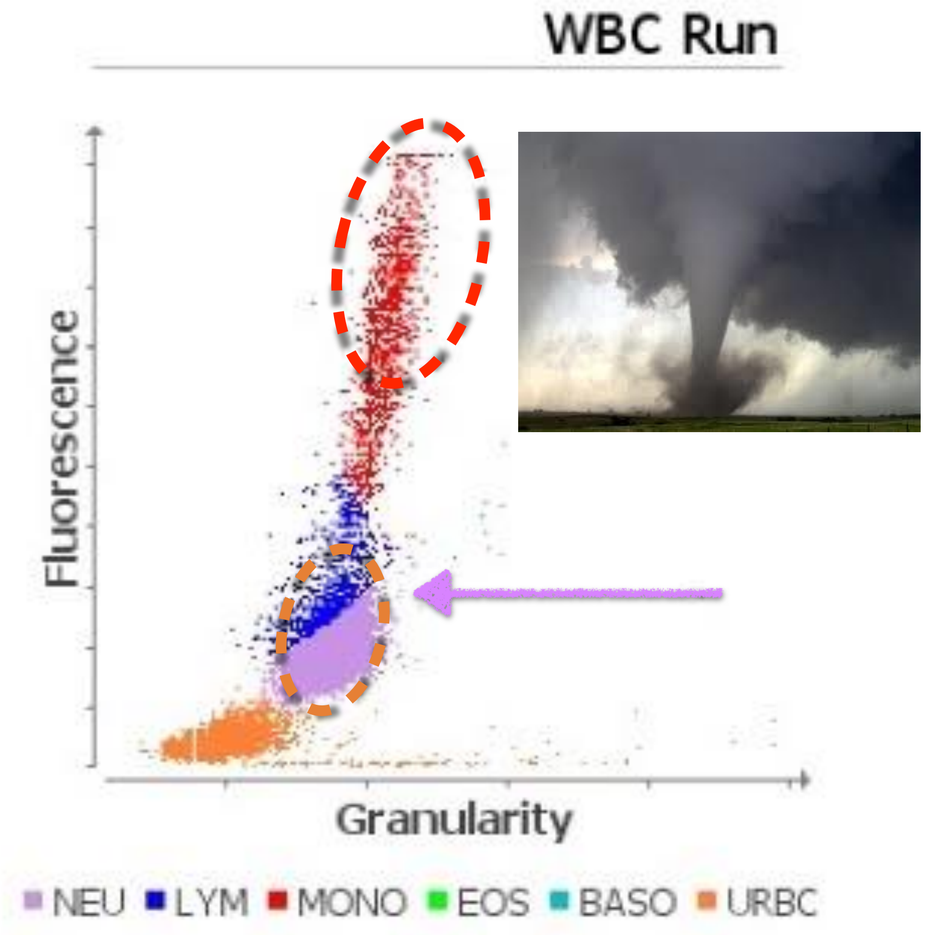
RBC Abnormal Distribution



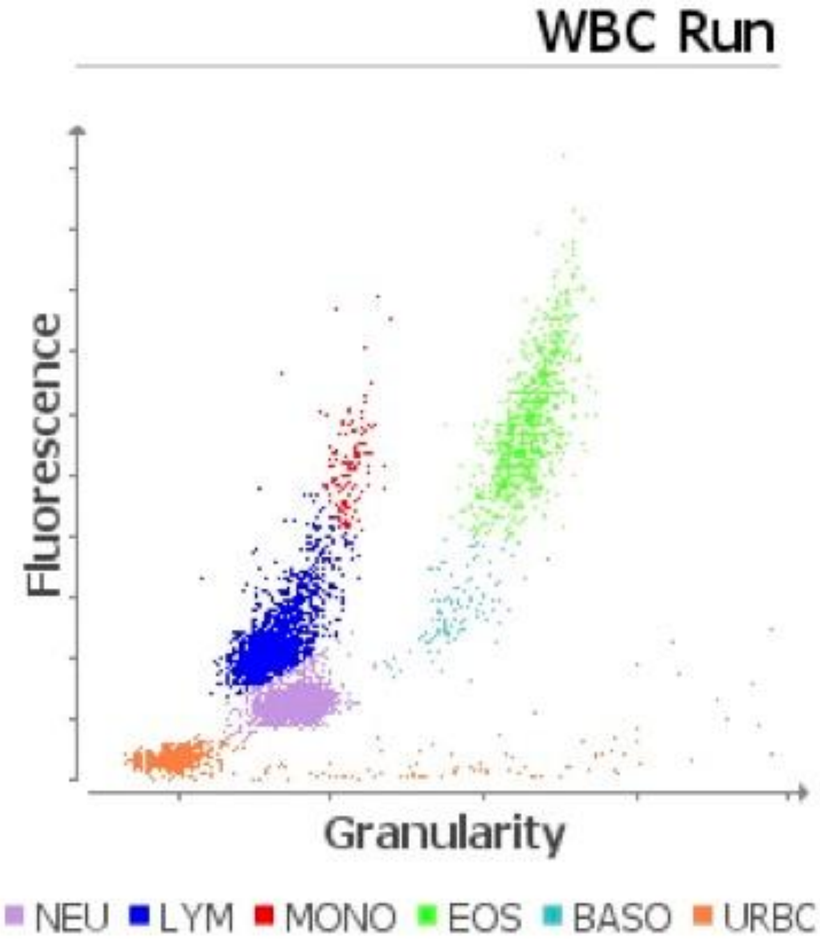


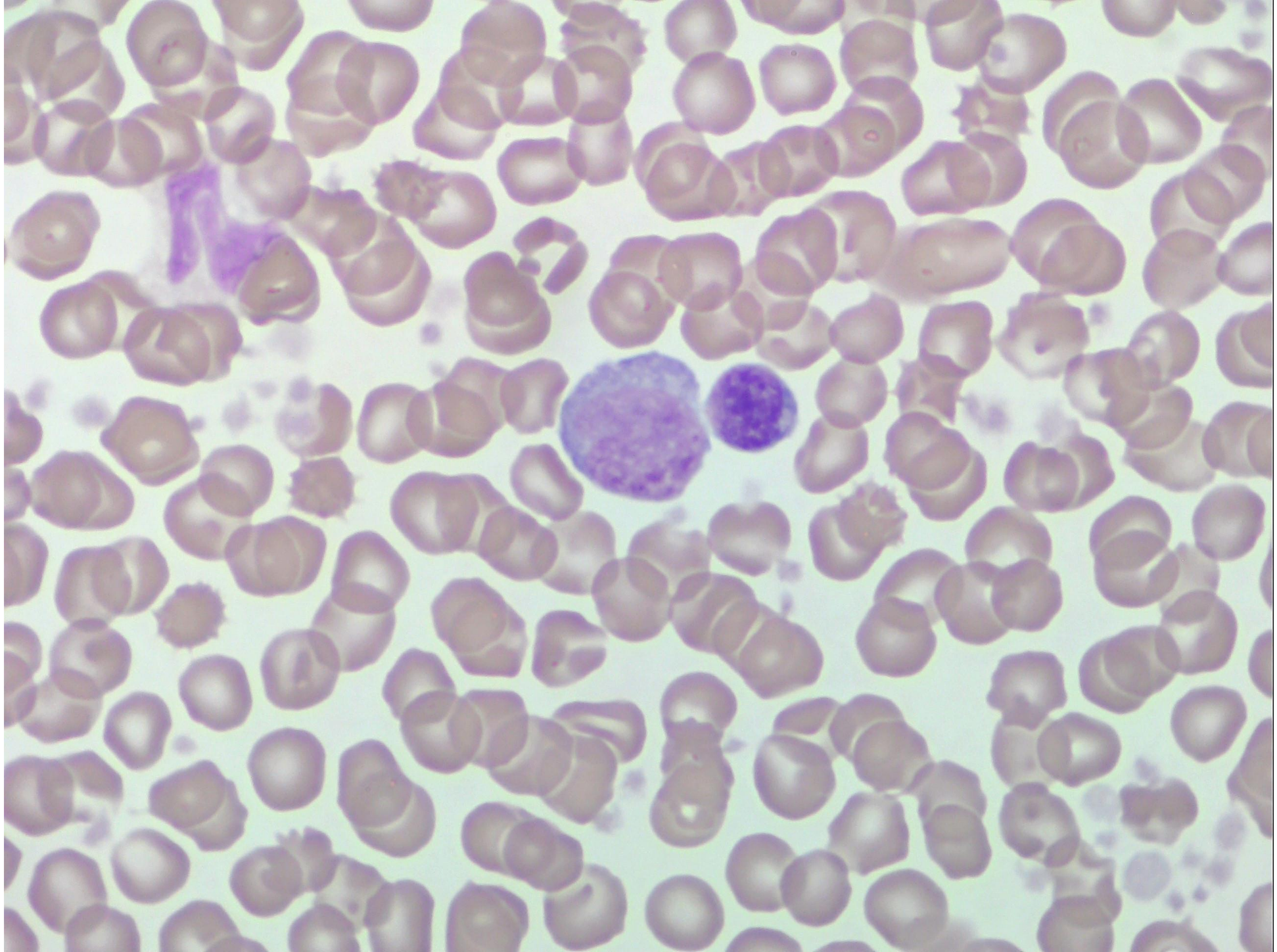
WBC	36.27	2.87 - 17.02 x10^9/L	
% Neutrophils	90.7	%	
% Lymphocytes	4.4	%	
% Monocytes	4.9	%	
% Eosinophils	0.0	%	
% Basophils	0.0	%	
Neutrophils	32.88	1.48 - 10.29 x10^9/L	
Lymphocytes	1.60	0.92 - 6.88 x10^9/L	
Monocytes	1.77	0.05 - 0.67 x10^9/L	
Eosinophils	0.01	0.17 - 1.57 x10^9/L	
Basophils	0.01	0.01 - 0.26 x10^9/L	

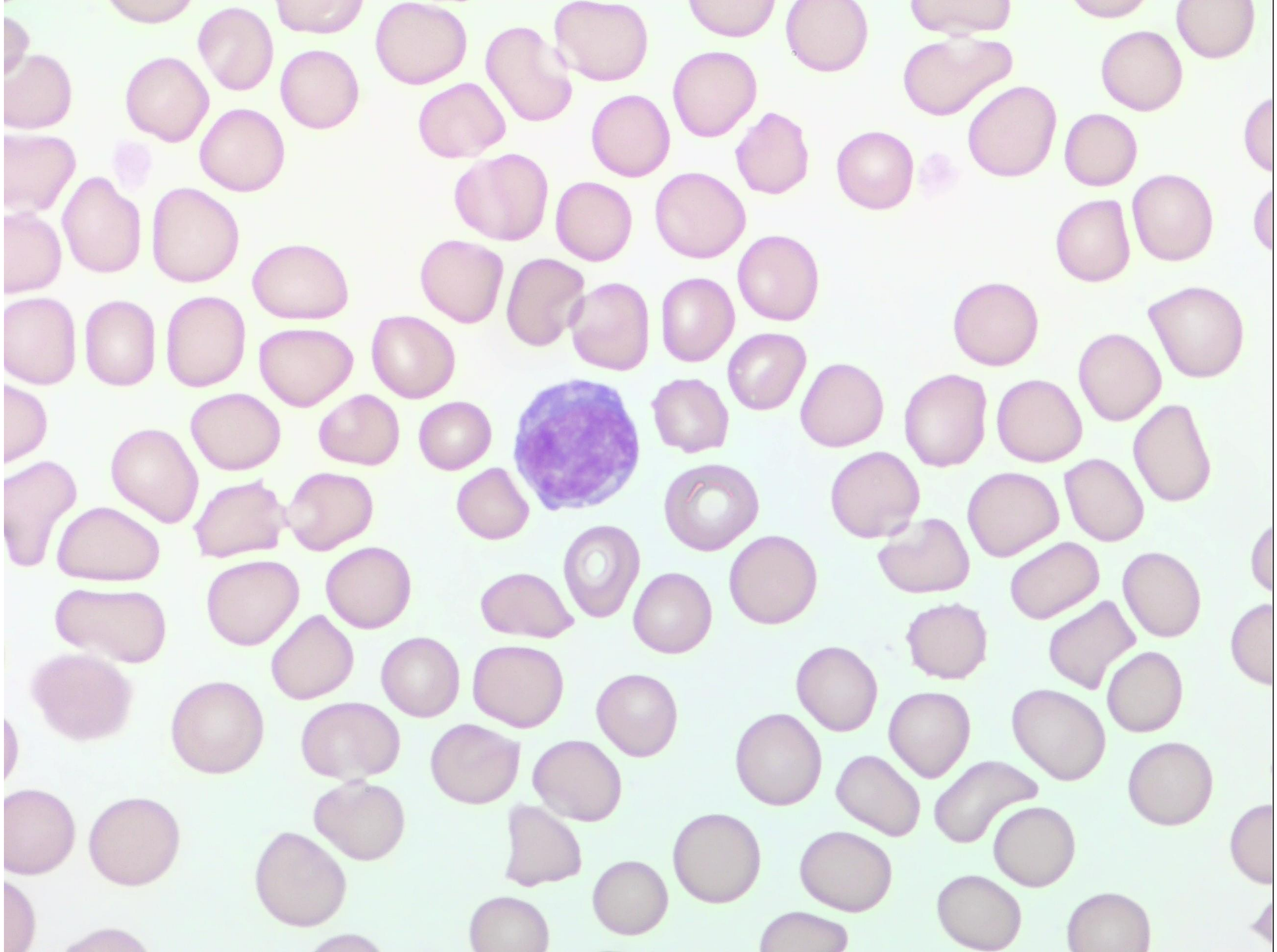
“Charlie”

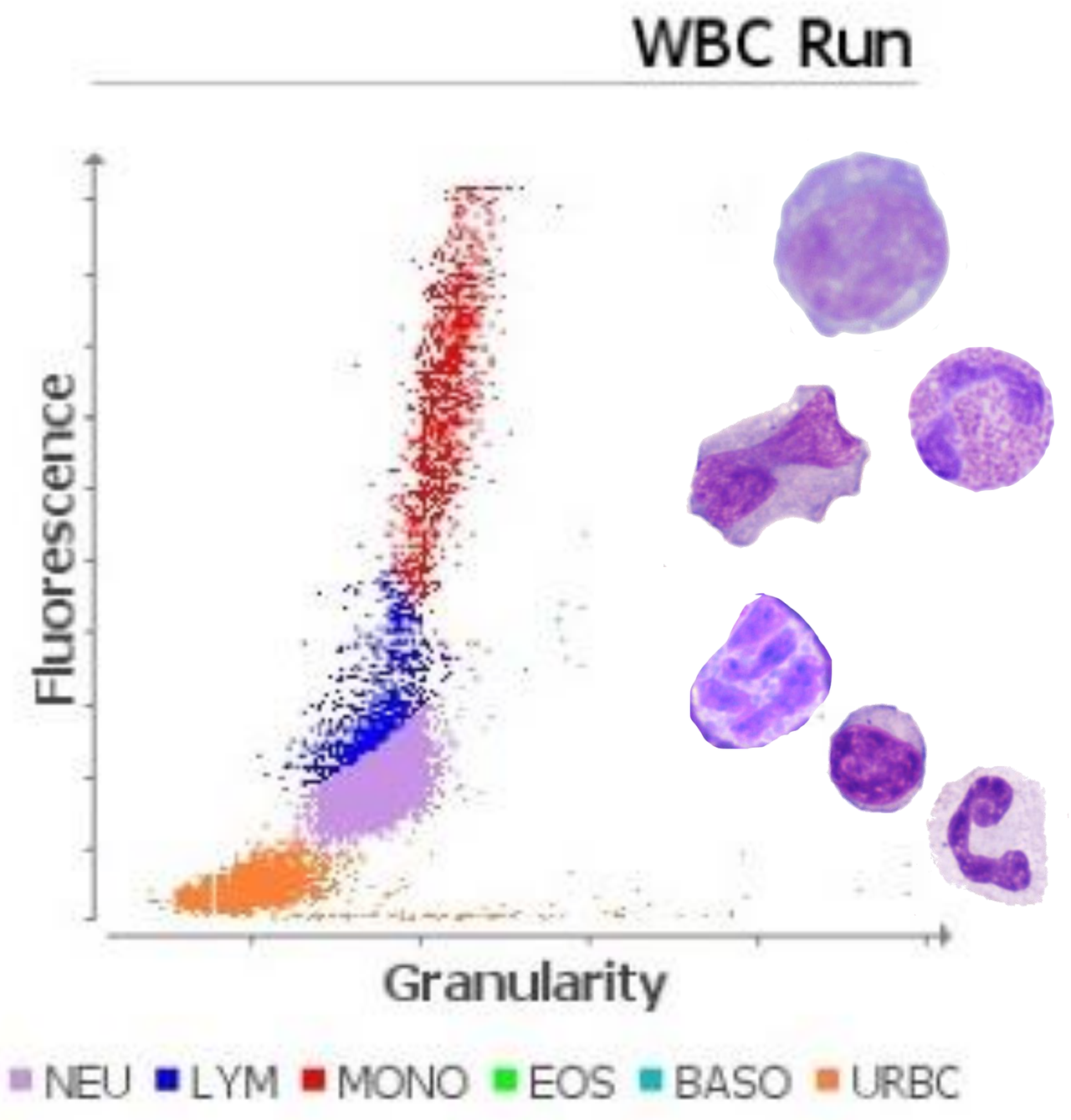


Normal





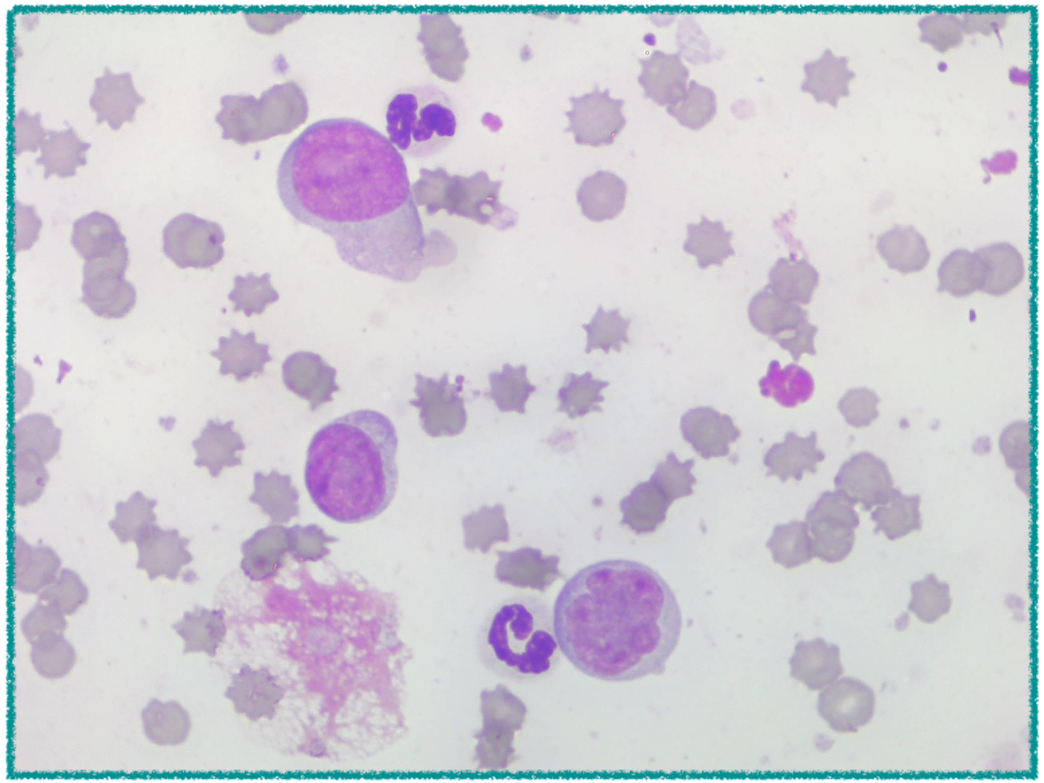



WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

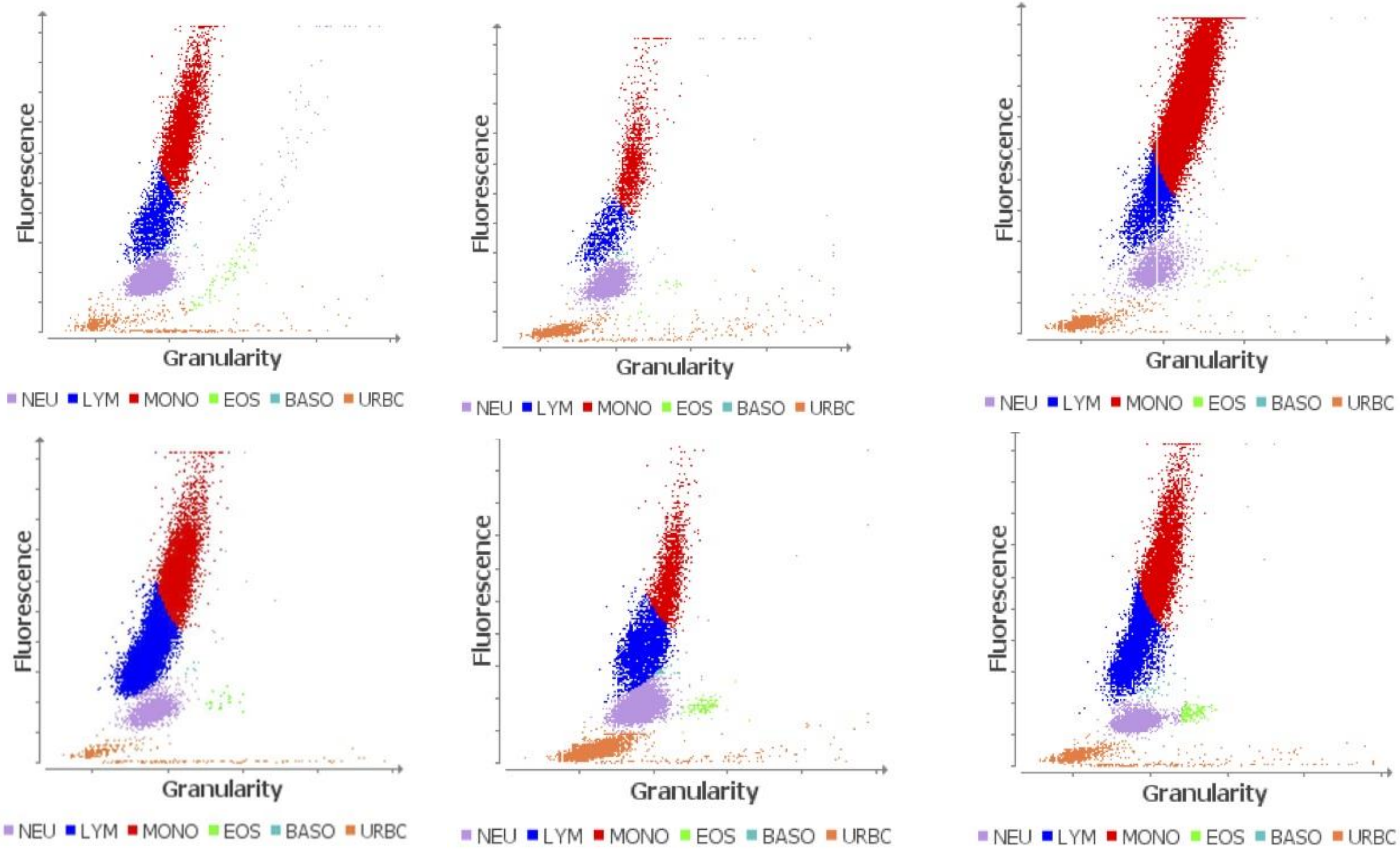
"Charlie"

FNA of the facial deformity: Lymphoma

Final Dx: Nasal lymphoma with circulating blasts



Lymphoma-Dogs

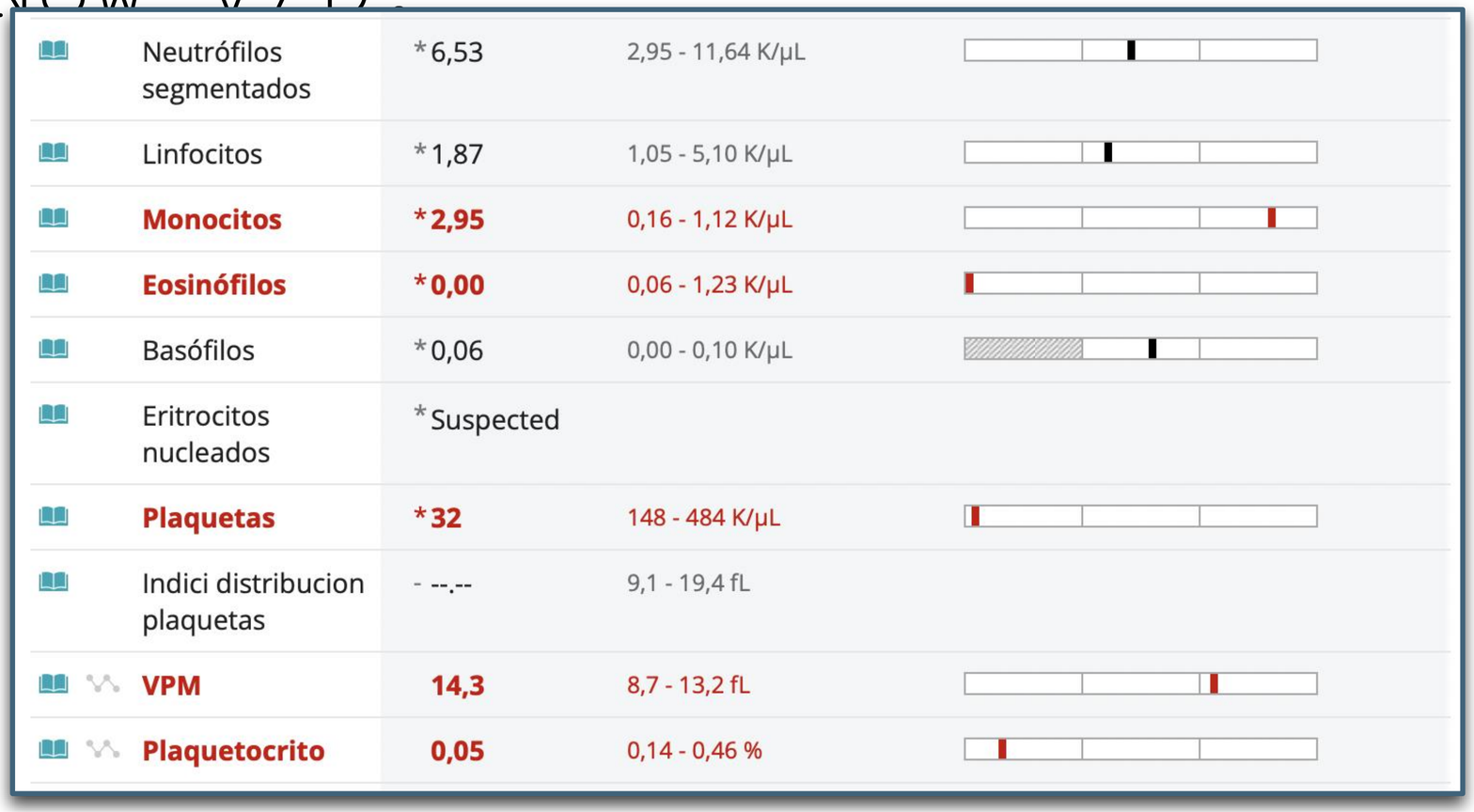


And with the ProCyte One?

Blanquita

Dr. Pachi Clemente

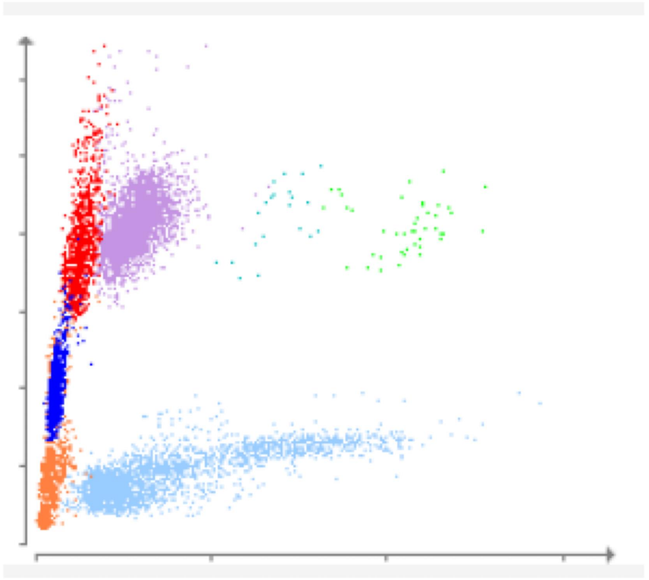
10, FS, Westie on chemo for large cell lymphoma. Now V/D.



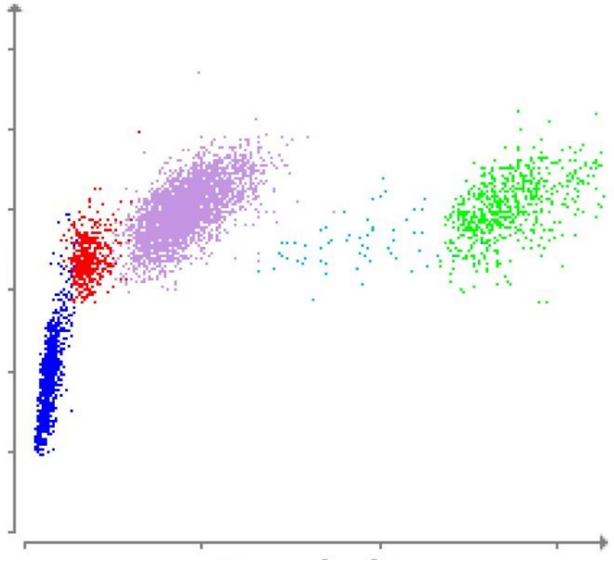
Neutrófilos segmentados	*6,53	2,95 - 11,64 K/µL	
Linfocitos	*1,87	1,05 - 5,10 K/µL	
Monocitos	***2,95**	0,16 - 1,12 K/µL	
Eosinófilos	***0,00**	0,06 - 1,23 K/µL	
Basófilos	*0,06	0,00 - 0,10 K/µL	
Eritrocitos nucleados	*Suspected		
Plaquetas	***32**	148 - 484 K/µL	
Indici distribucion plaquetas	- --.--	9,1 - 19,4 fL	
VPM	**14,3**	8,7 - 13,2 fL	
Plaquetocrito	**0,05**	0,14 - 0,46 %	

Blanquita

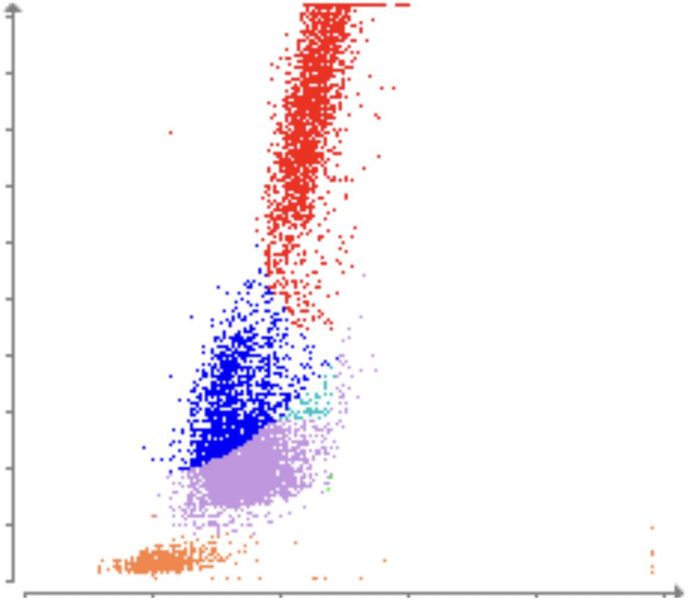
Dr. Pachi Clemente



ProCyte One-Blanquita



ProCyte One-Normal

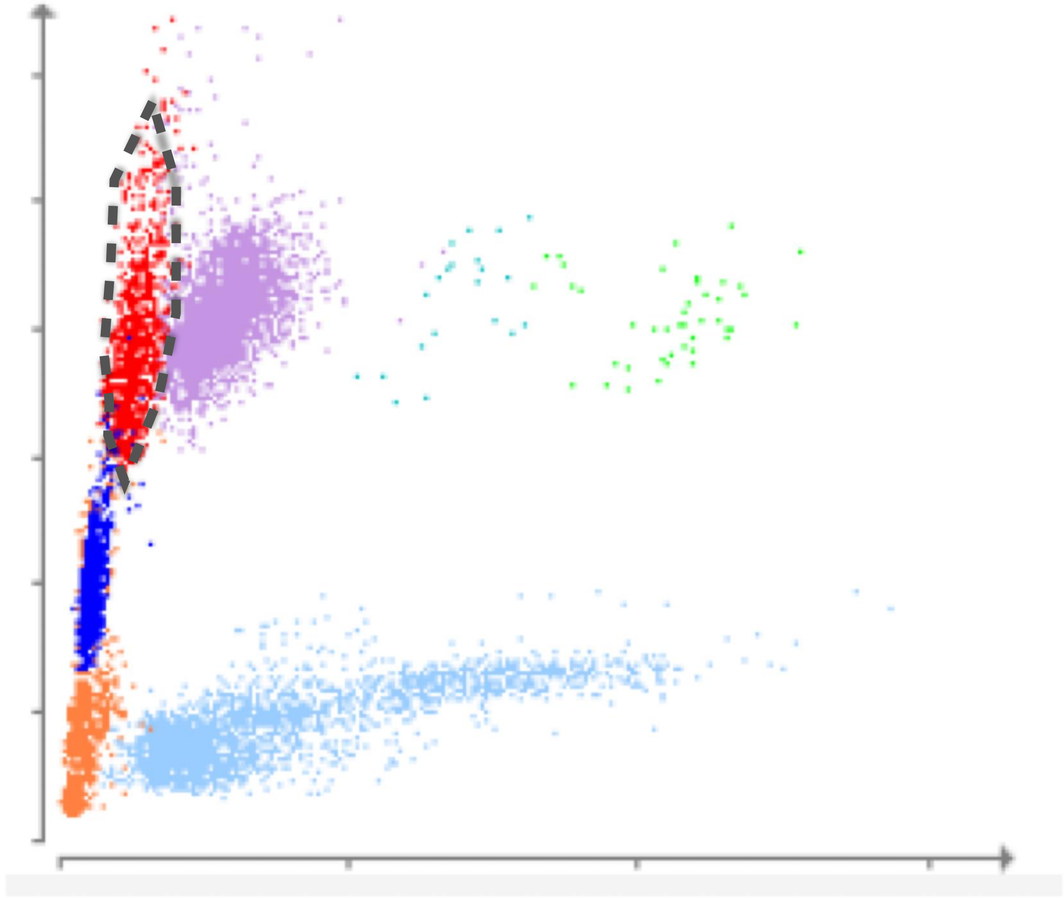


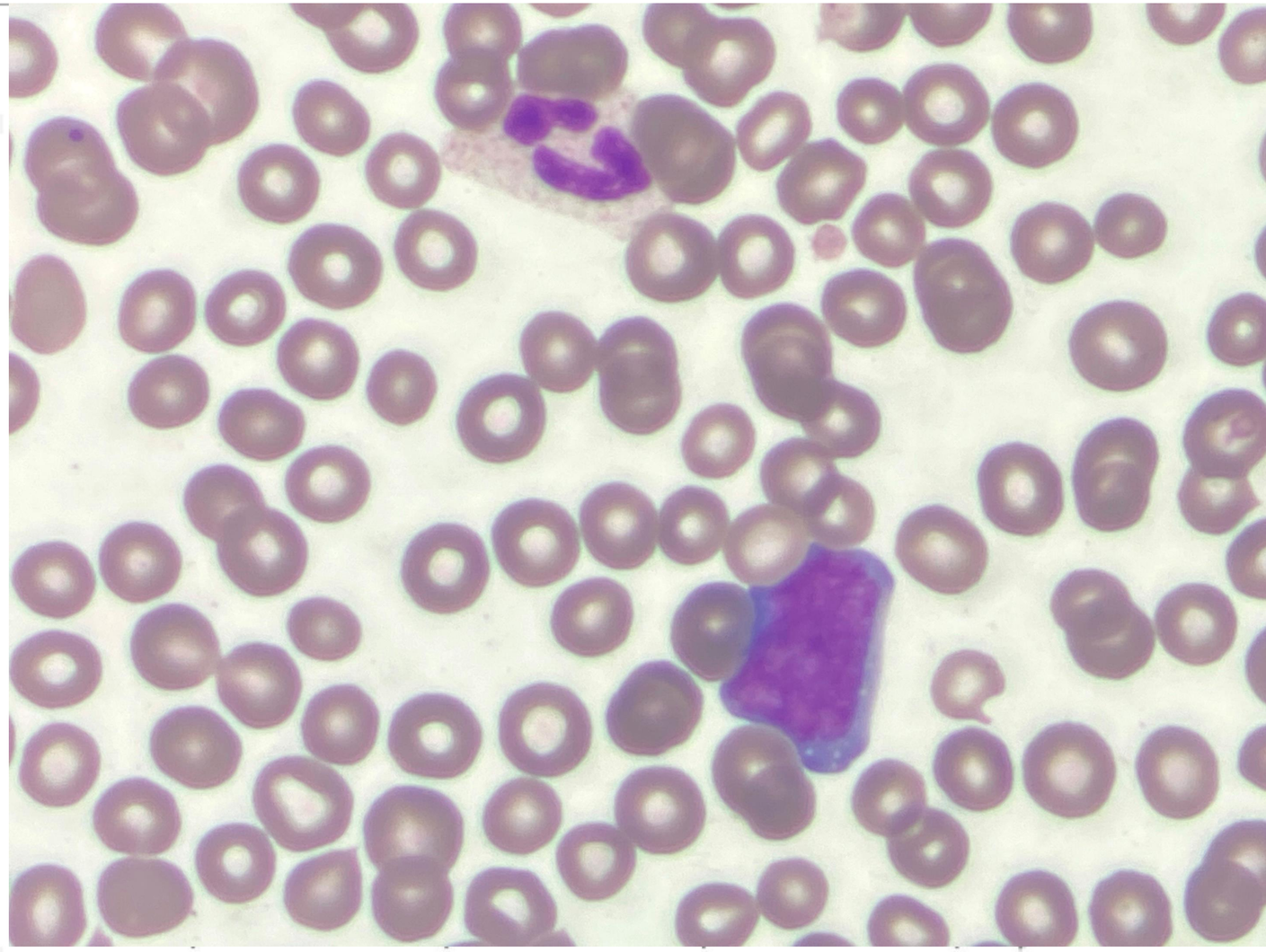
ProCyte Dx-Blanquita

Blanquita

Dr. Pachi Clemente

ProCyte One-Blanquita





PCDx and Lymphoma

Now lots of dogs and cats “become” stage V!

Does stage migration affect prognosis?

Not likely for most…

EXAMPLE: T-zone lymphoma patients frequently have circulating tumor cells

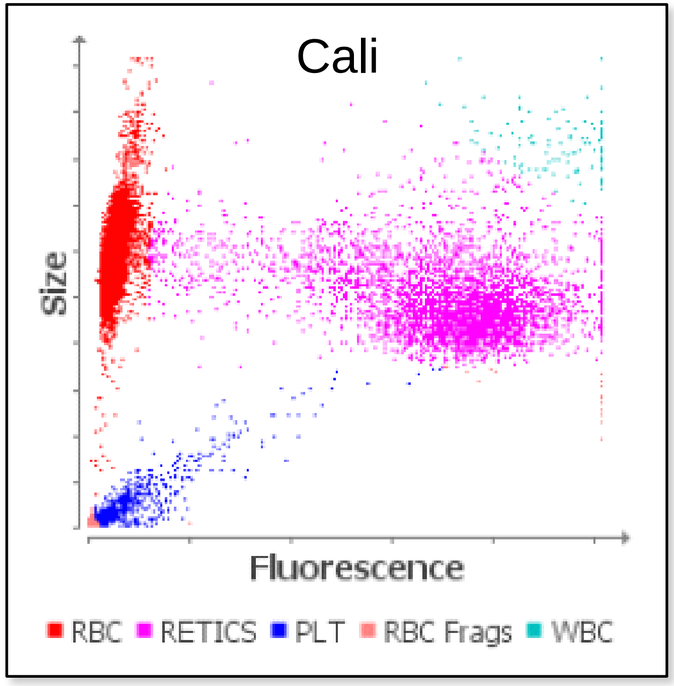
Cali

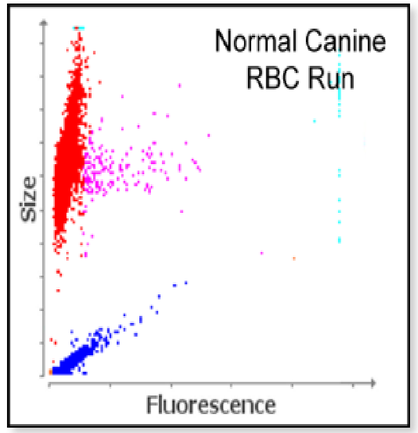
- 11 year old
- Female spayed
- Golden Retriever

- Routine health check
- Generalized lymphadenopathy

Cali – RBC

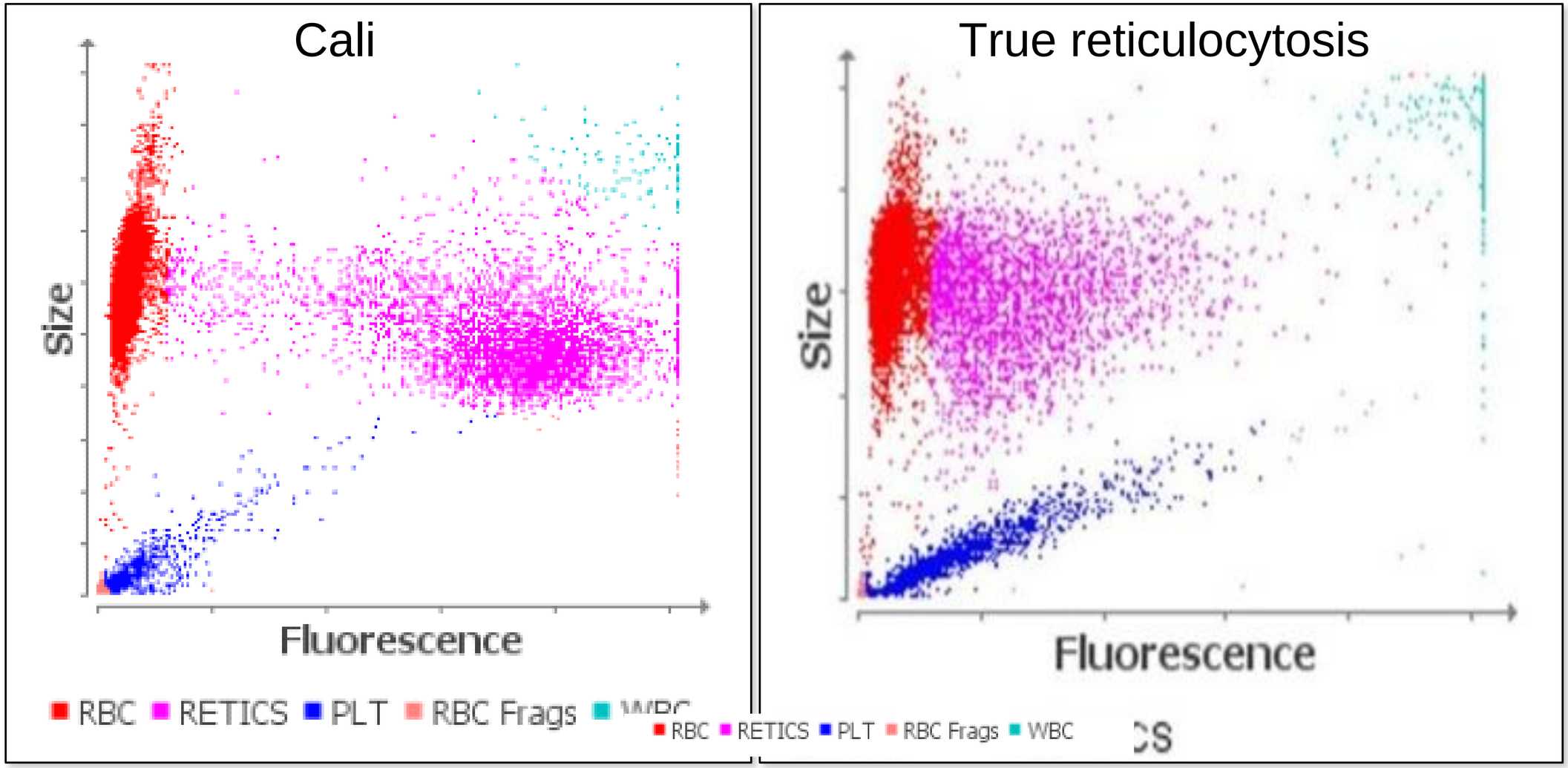
RBC	2.89	5.65 - 8.87 x10^12/L	
Haematocrit	0.212	0.373 - 0.617 L/L	
Haemoglobin	76	131 - 205 g/L	
MCV	73.4	61.6 - 73.5 fL	
MCH	26.3	21.2 - 25.9 pg	
MCHC	358	320 - 379 g/L	
RDW	14.9	13.6 - 21.7 %	
% Reticulocytes	19.3	%	
Reticulocytes	558.6	10.0 - 110.0 K/µL	





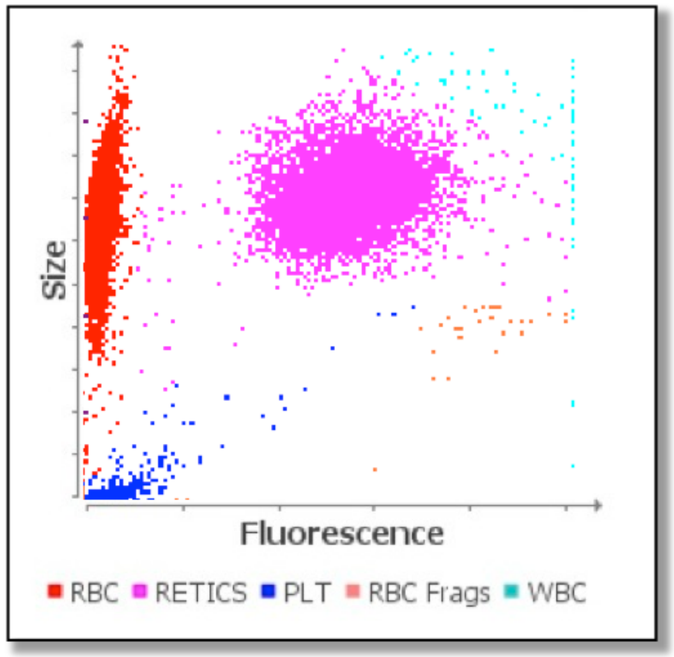
Regenerative or nonregenerative ?

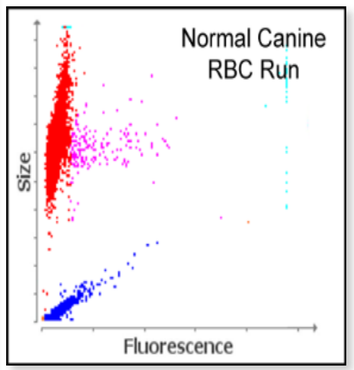
Cali - RBCs


Cali
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
True reticulocytosis
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

Lolle – RBC

Test	Results	Reference Interval	LOW	NORMAL	HIGH
ProCyte Dx					
RBC	2.05 M/µL	5.65 - 8.87	LOW		
HCT	15.5 %	37.3 - 61.7	LOW		
HGB	5.0 g/dL	13.1 - 20.5	LOW		
MCV	75.6 fL	61.6 - 73.5	HIGH		
MCH	24.4 pg	21.2 - 25.9			
MCHC	32.3 g/dL	32.0 - 37.9			
RDW	18.6 %	13.6 - 21.7			
%RETIC	26.3 %				
RETIC	538.9 K/µL	10.0 - 110.0	HIGH		



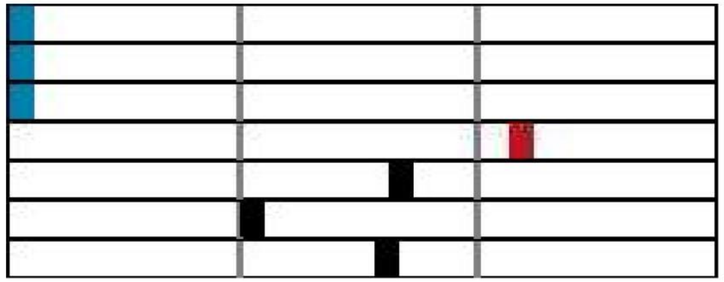


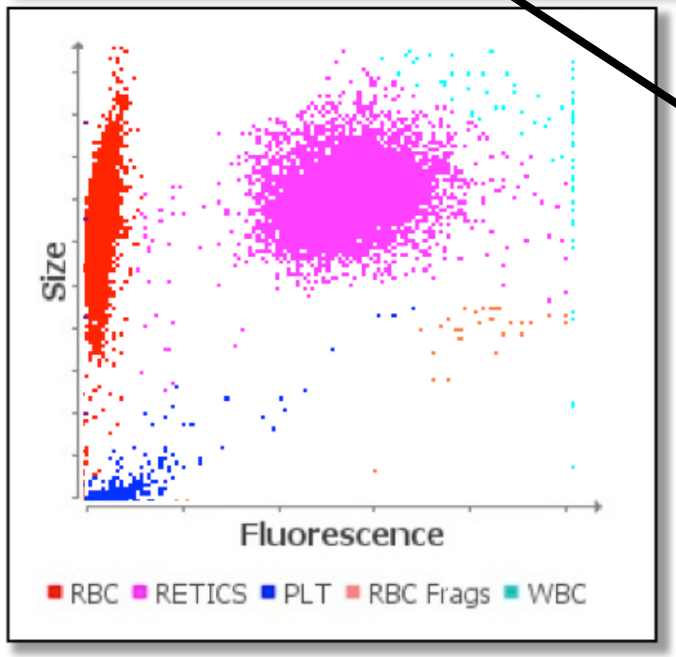
Reported marked reticulocytosis

Regenerative or nonregenerative ?

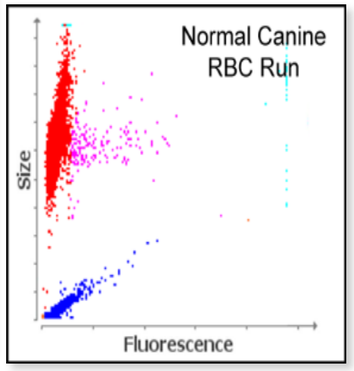
Lolle

Test	Results	Reference Interval	LOW	NORMAL	HIGH
ProCyte Dx					
RBC	2.05 M/µL	5.65 - 8.87	LOW		
HCT	15.5 %	37.3 - 61.7	LOW		
HGB	5.0 g/dL	13.1 - 20.5	LOW		
MCV	75.6 fL	61.6 - 73.5	HIGH		
MCH	24.4 pg	21.2 - 25.9			
MCHC	32.3 g/dL	32.0 - 37.9			
RDW	18.6 %	13.6 - 21.7			
%RETIC	--.-- %				
RETIC	--.-- K/µL	10.0 - 110.0			

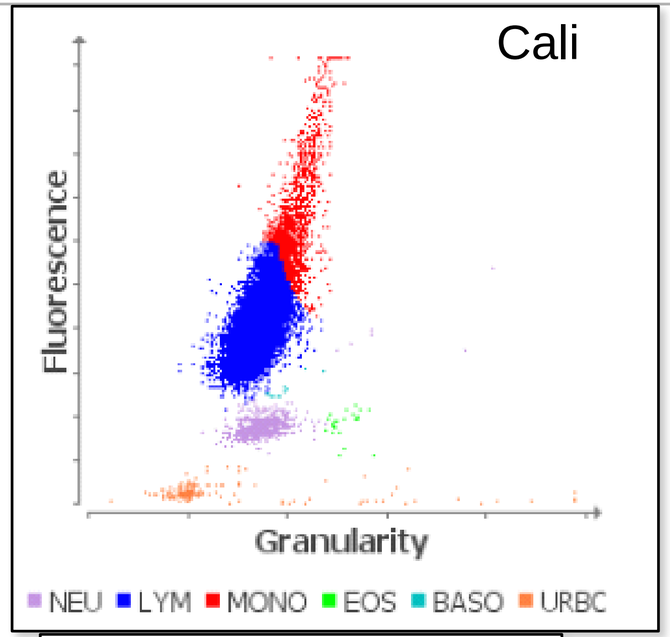


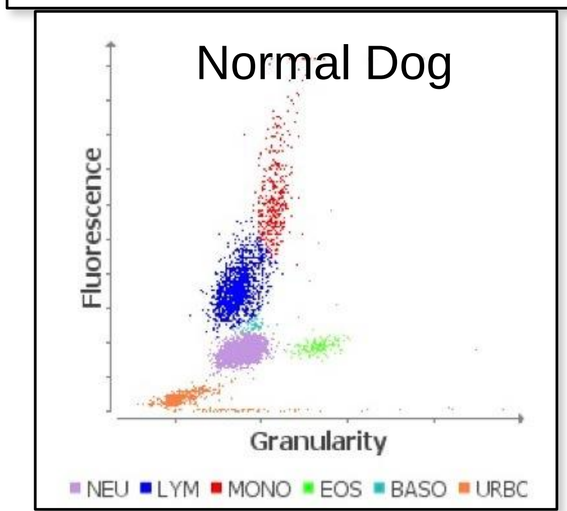


Current software – pseudoreticulocytosis
No reticulocyte count reported



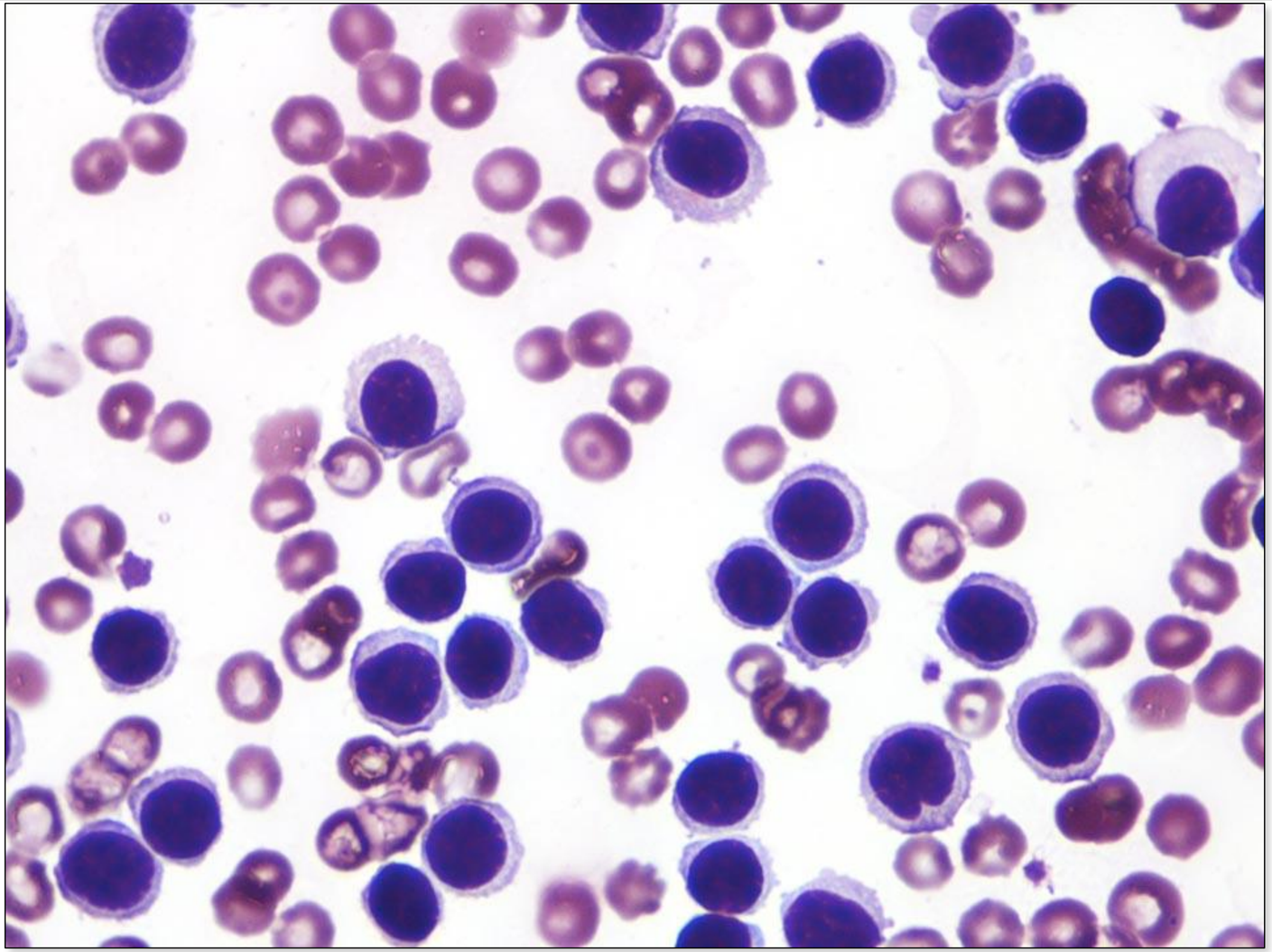
Cali - WBCs





WBC	***621.97**	5.05 - 16.76 x10^9/L	
% Neutrophils	*2.5	%	
% Lymphocytes	*92.0	%	
% Monocytes	*5.4	%	
% Eosinophils	0.1	%	
% Basophils	0.0	%	
Neutrophils	***15.95**	2.95 - 11.64 x10^9/L	
Bands	*Suspect presence		
Lymphocytes	***571.98**	1.05 - 5.10 x10^9/L	
Monocytes	***33.35**	0.16 - 1.12 x10^9/L	
Eosinophils	0.44	0.06 - 1.23 x10^9/L	
Basophils	**0.25**	0.00 - 0.10 x10^9/L	

Cali – Blood Film



Cali – Flow Cytometry

Immunophenotyping data:
The cells in the lymphocyte gate are small to intermediate in size and correlate with the lymphocyte population described in the pathologist review of the blood smear. On flow cytometry, the lymphocytes express the following markers:

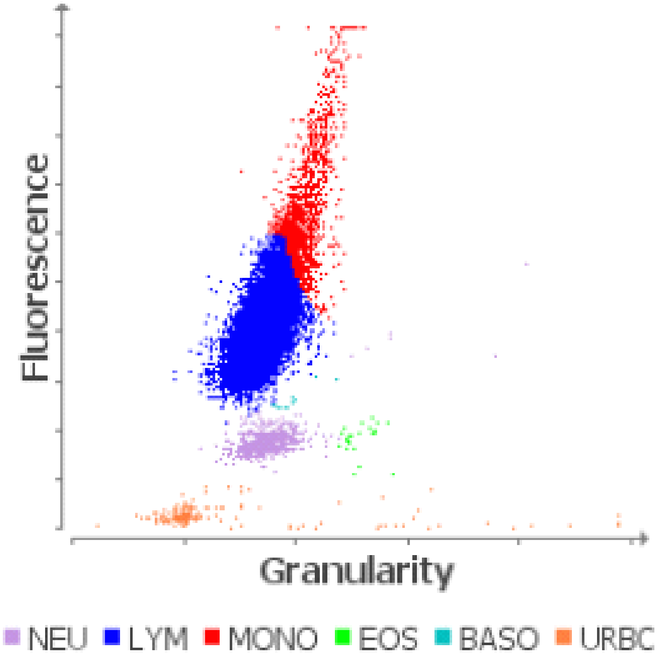
T-cell markers:
99% of the cells express CD3 and CD5. These are both pan T lymphocyte markers. In the T-cell population, less than 1% of these are CD4 positive T-helper cells, and less than 1% are CD8 positive cytotoxic T-cells. The remaining T lymphocytes (98% of the cells) are double negative (do not express either CD4 or CD8).

B-cell marker:
Less than 5% of the cells express CD21.

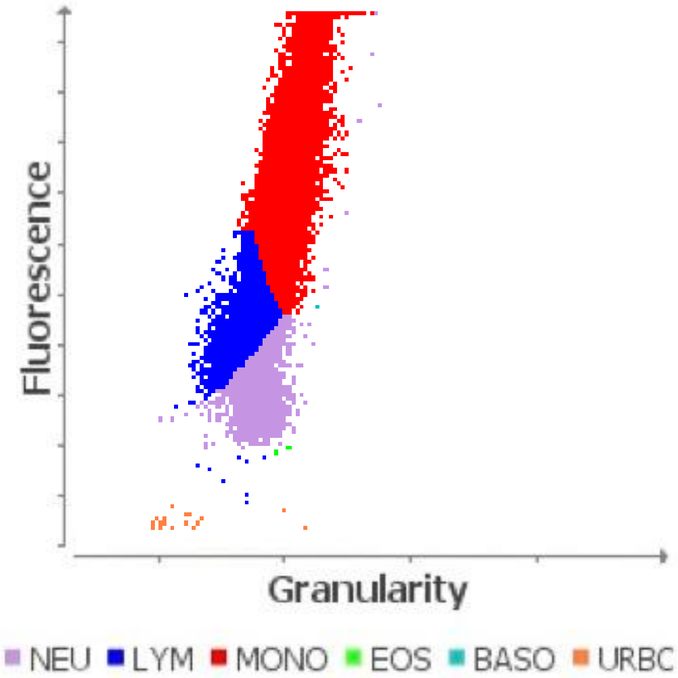
Final diagnosis: Chronic lymphocytic leukemia (T-cell)

- 99% of neoplastic cells express CD3 and CD5
- Pan T lymphocyte markers

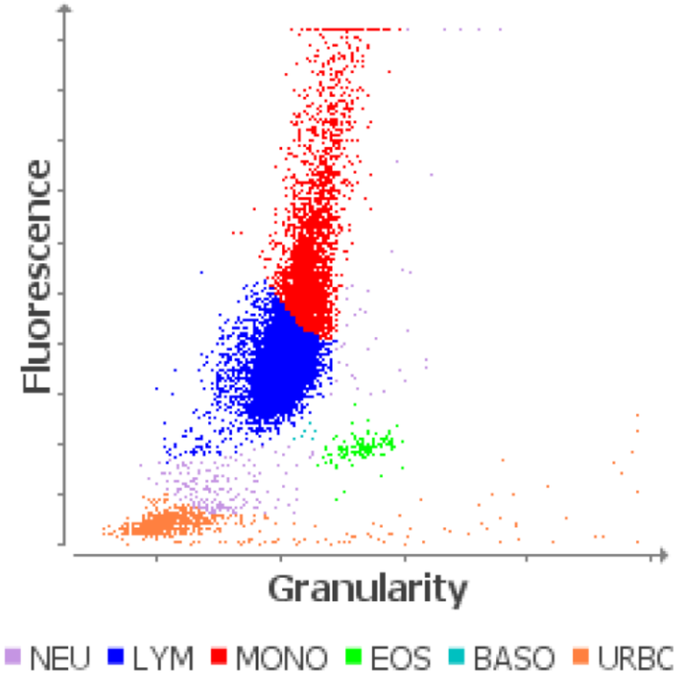
CLL



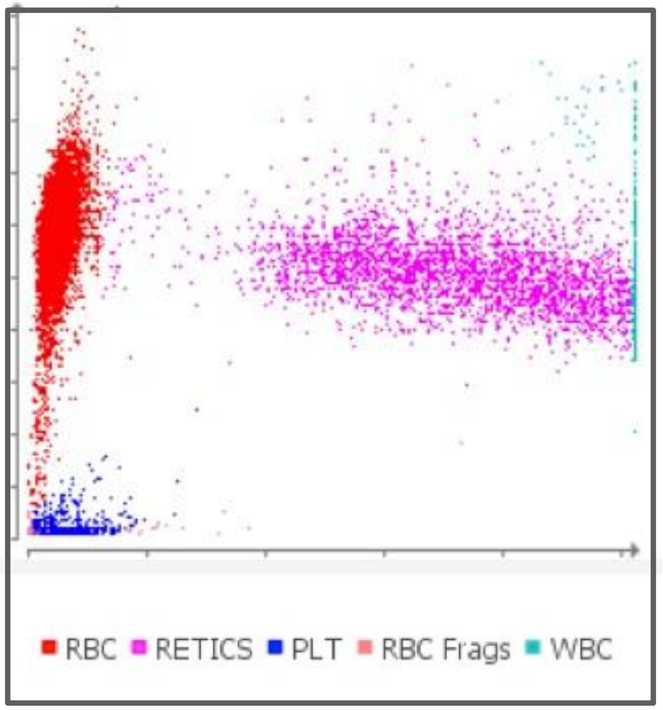
ALL



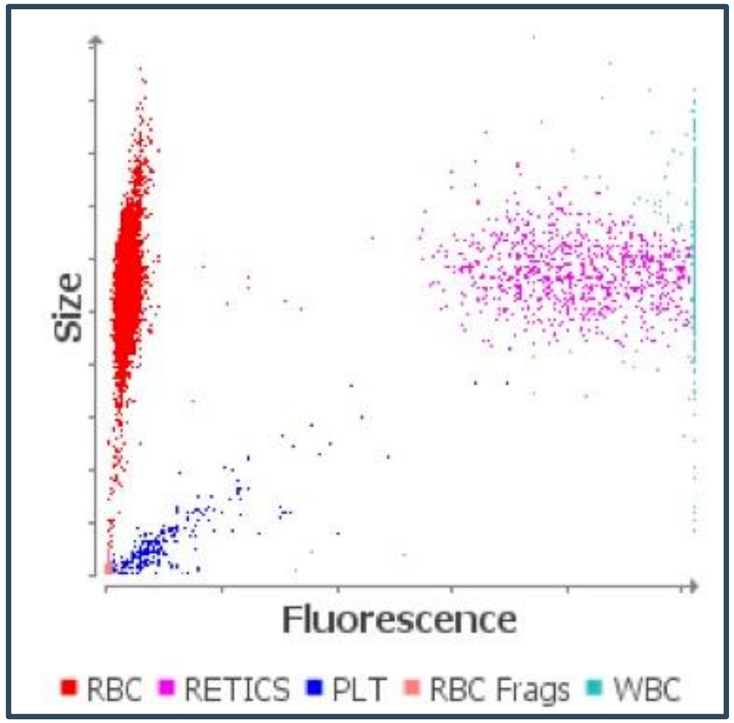
Marked Left Shift



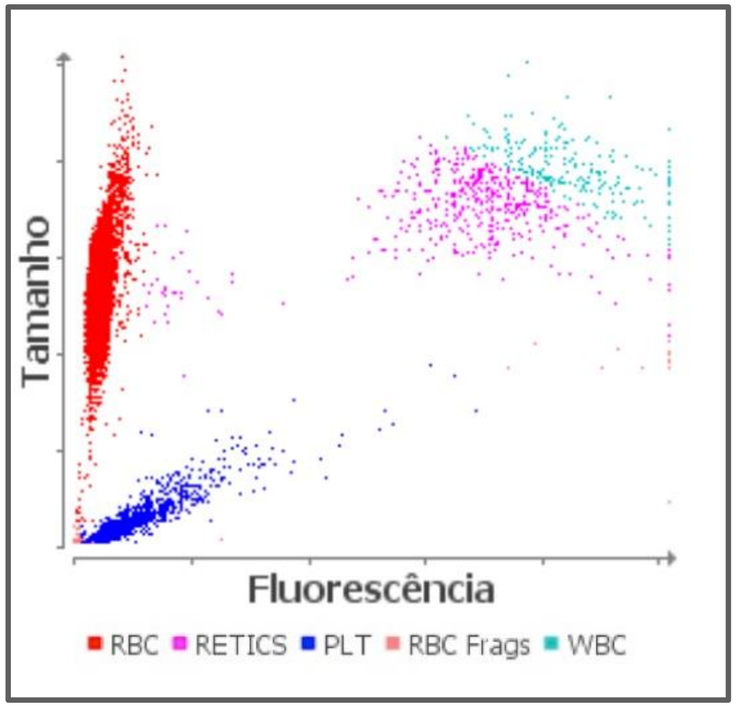
CLL



ALL



Pyometra



Conclusions

Please don't just look at the numbers

If you use a ProCyte, graphics are invaluable!

Keep an eye on those "monocytes"!

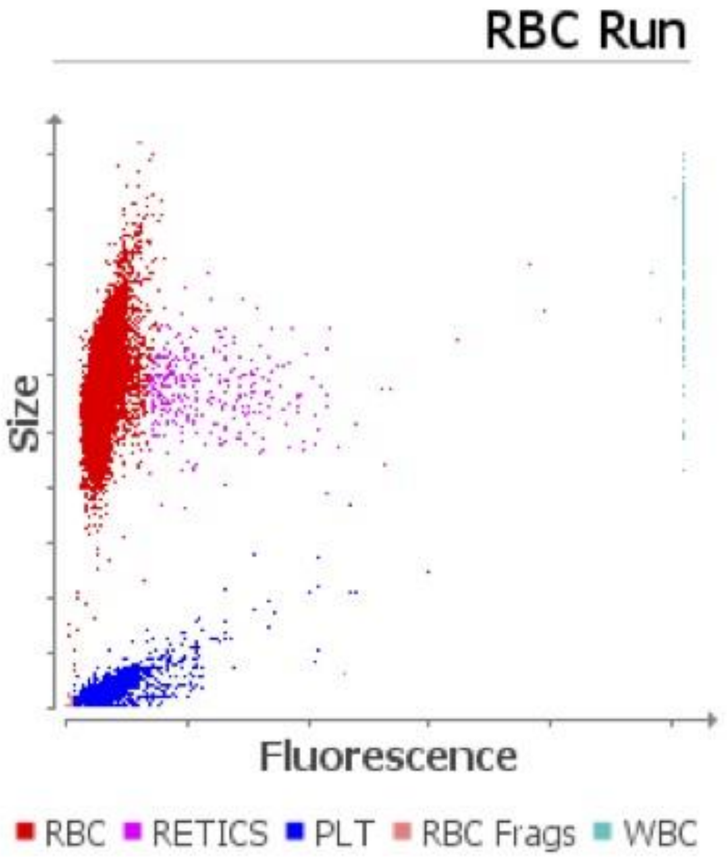
coutovetconsultants@gmail.com

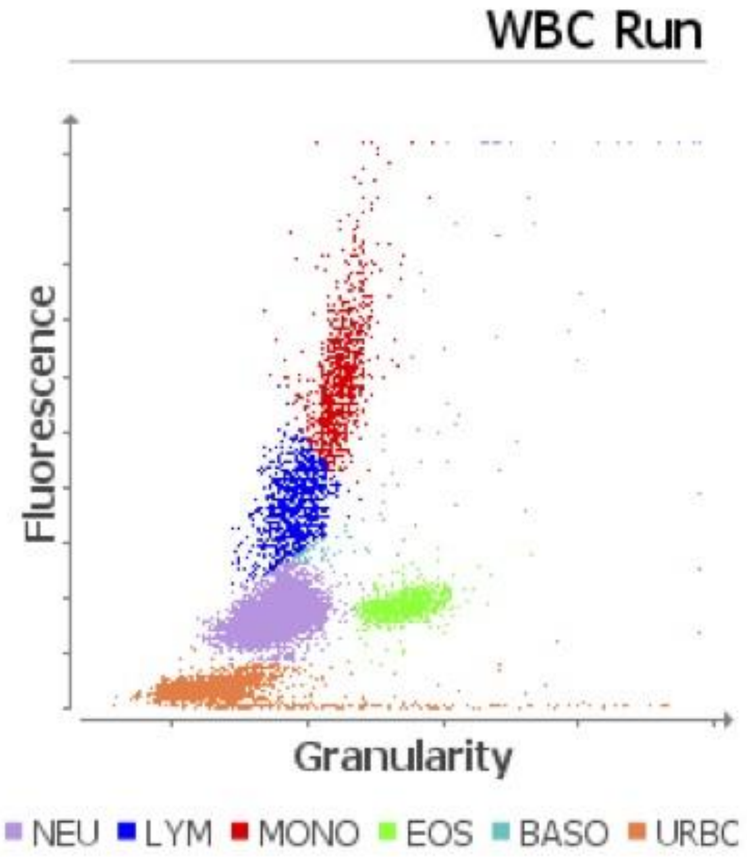
“Pudge”

13-year-old, MC, Shepherd mix with multicentric lymphoma on CHOP

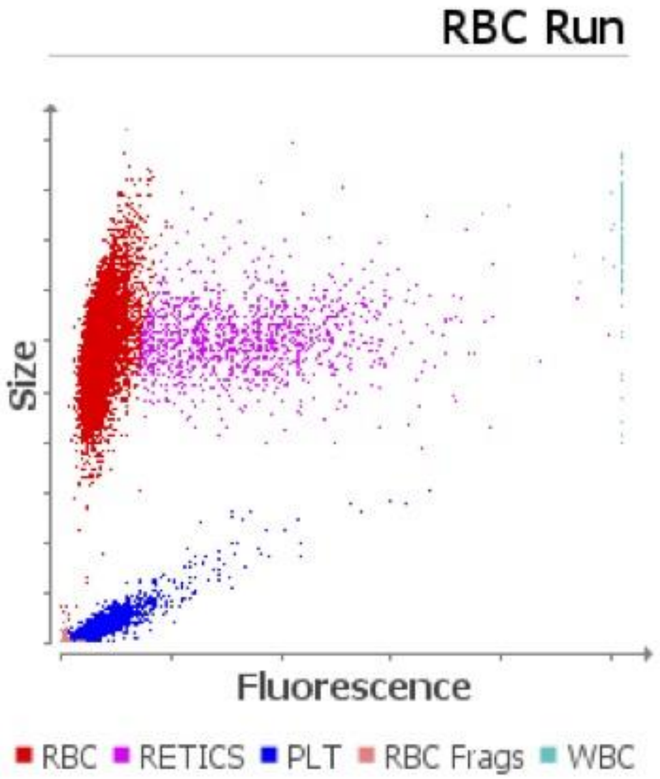
Let the dot plots tell a story…

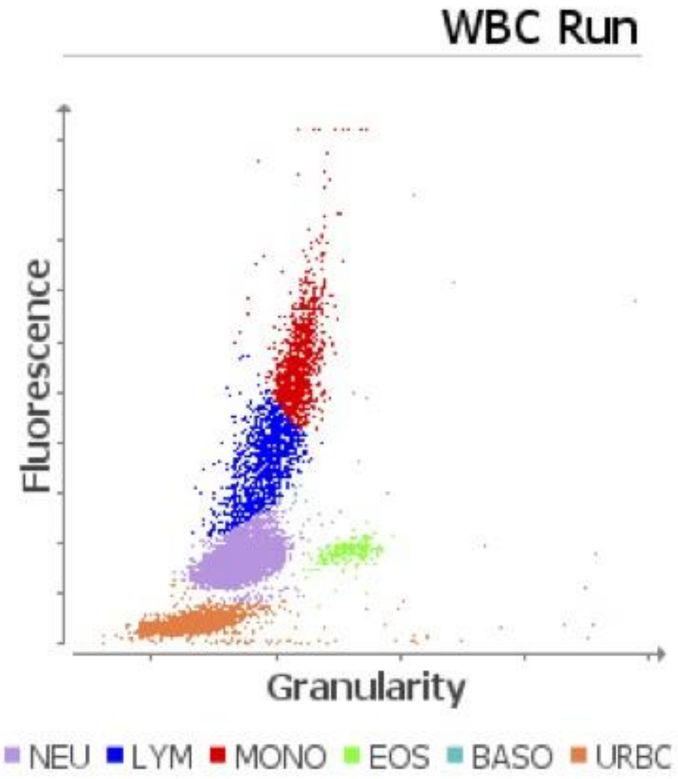
Day 1


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

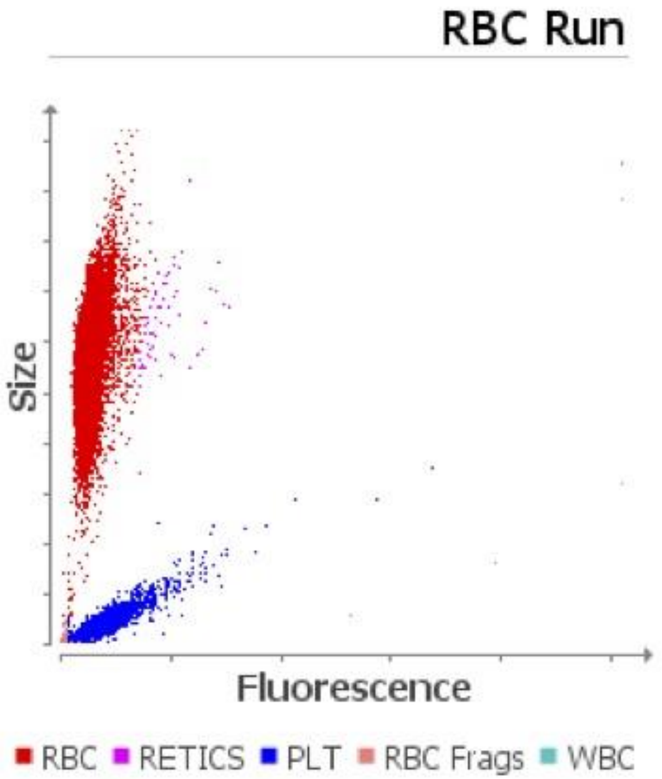

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

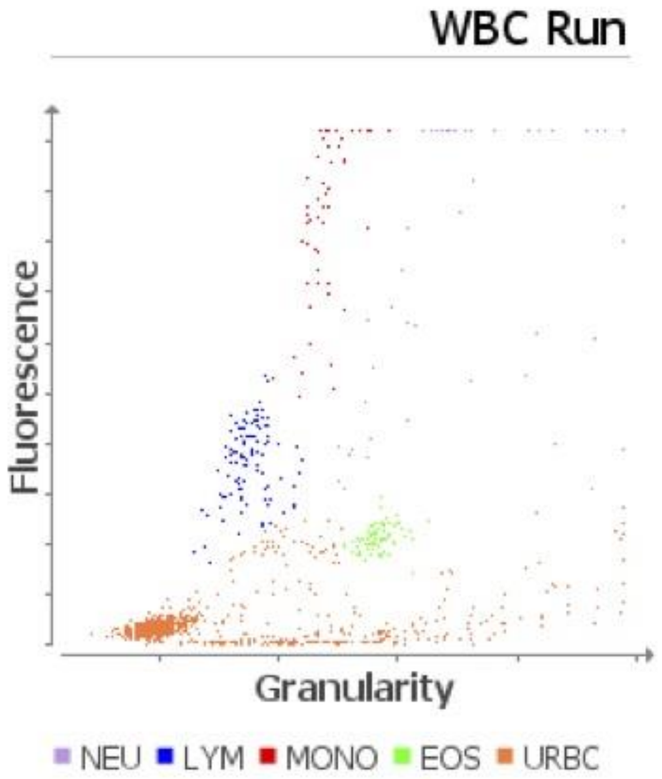
Day 8


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

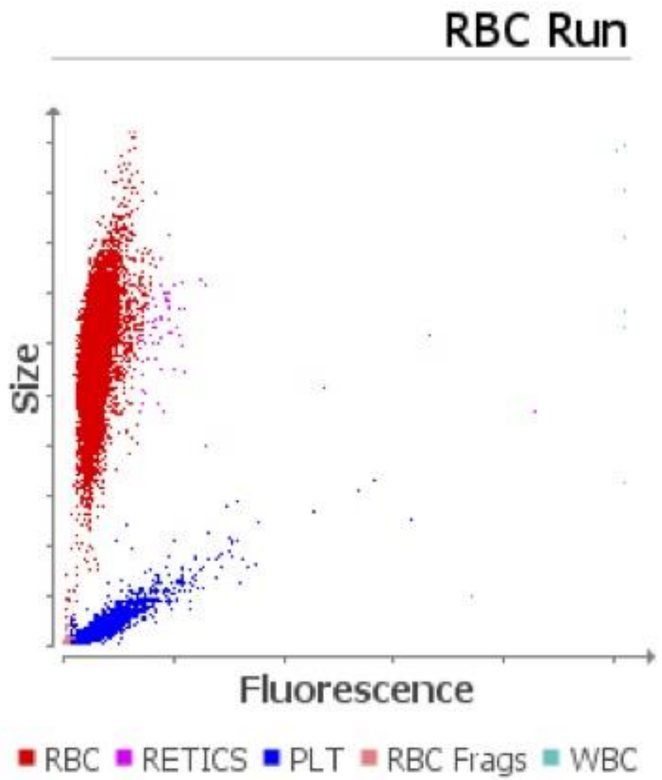

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

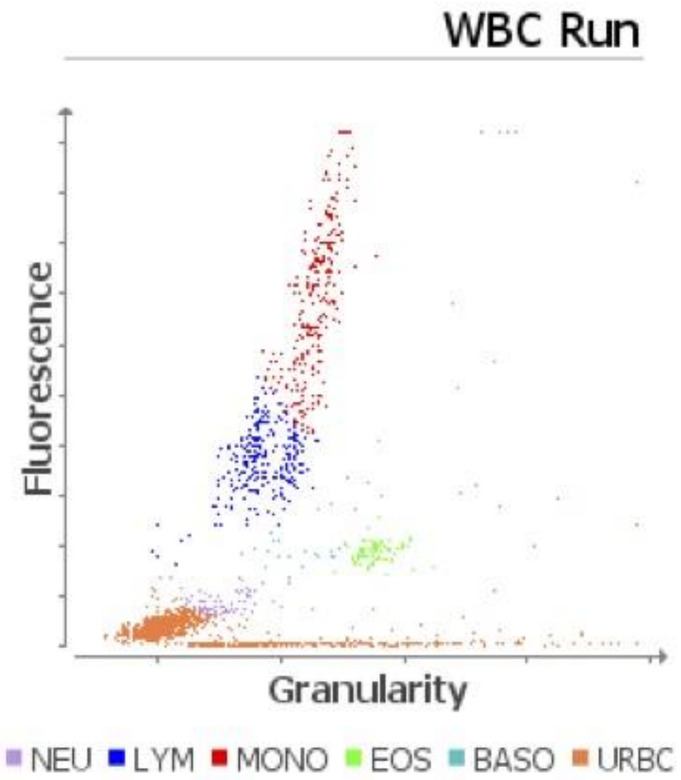
Day 30


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

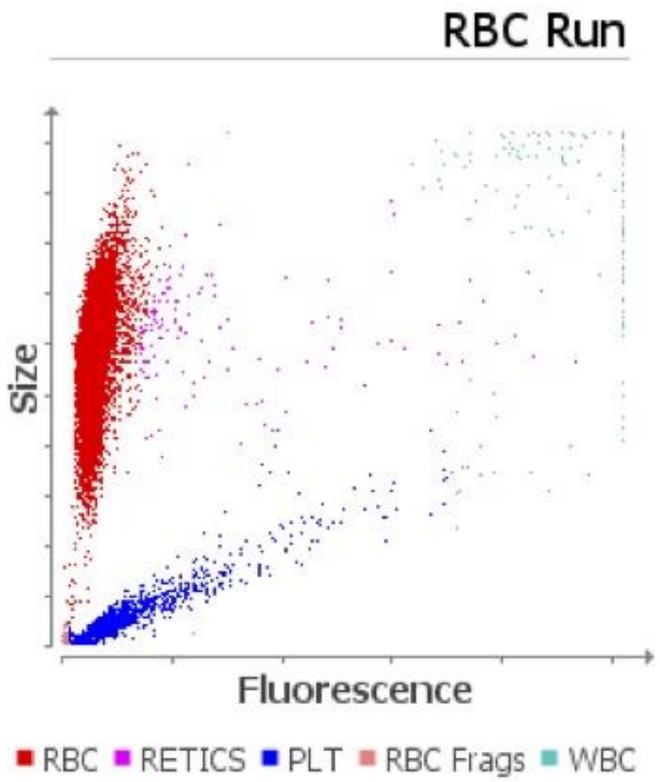

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
URBC

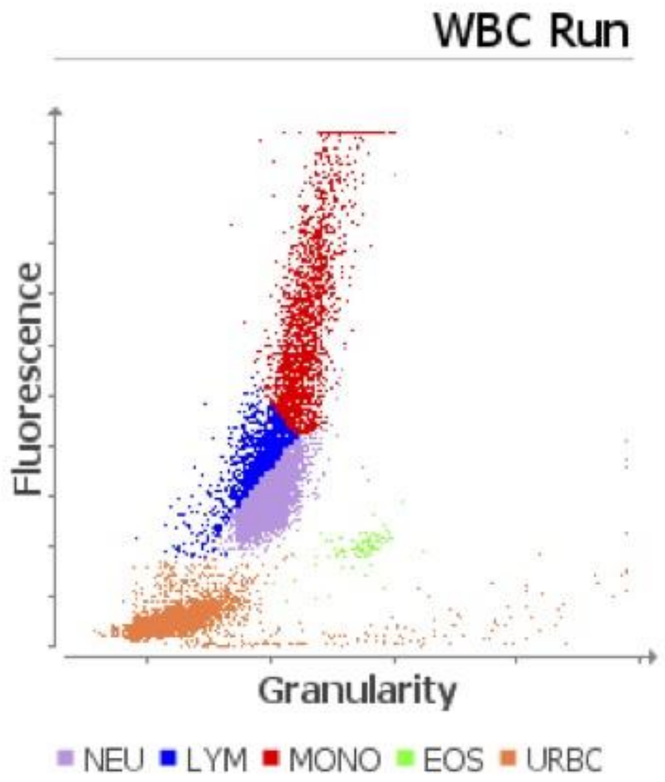
Day 31


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

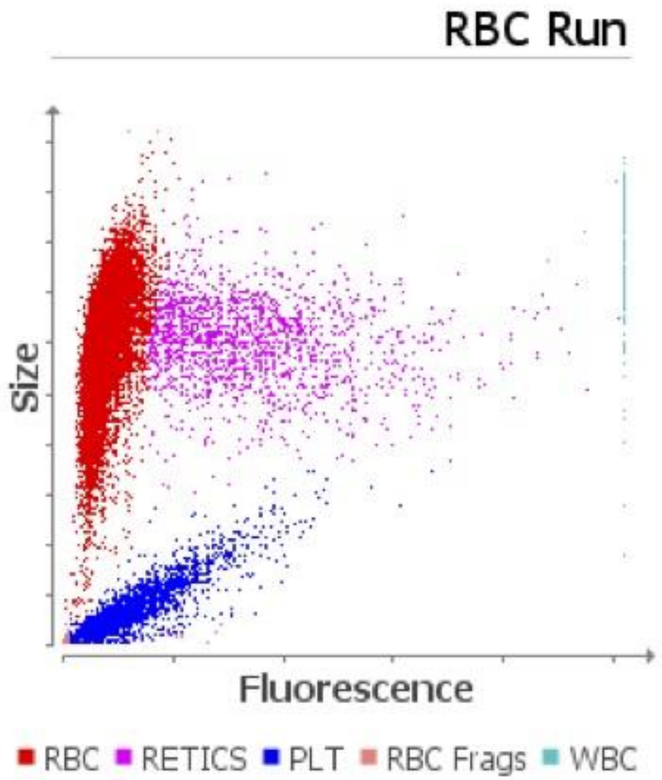

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

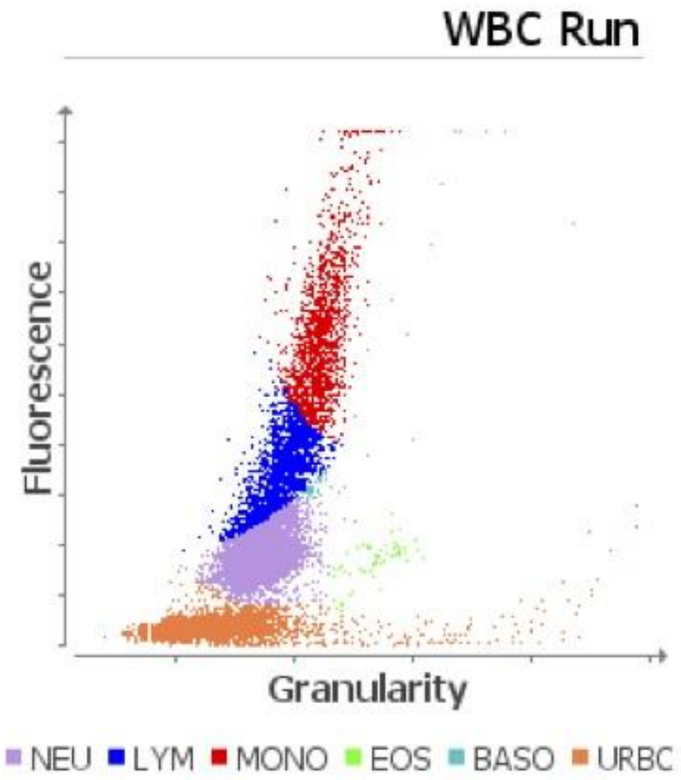
Day 32


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

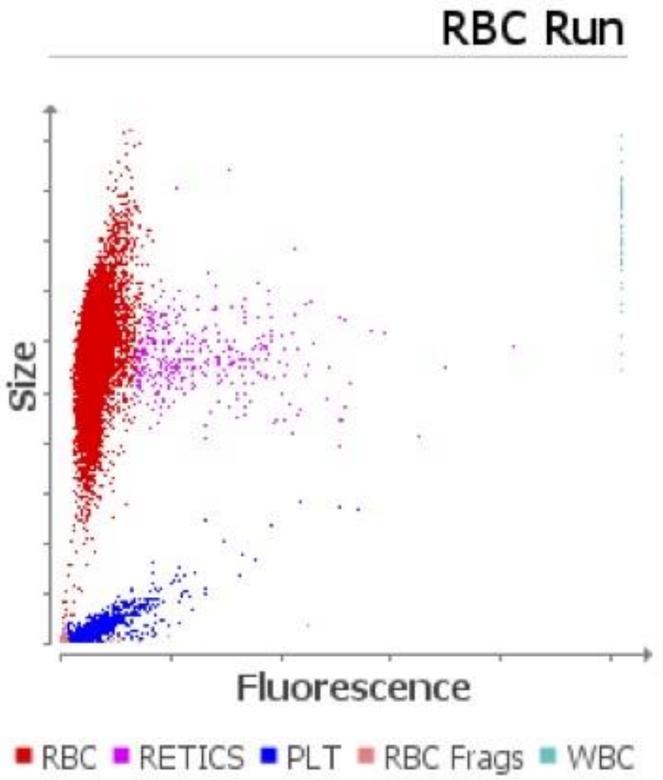

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
URBC

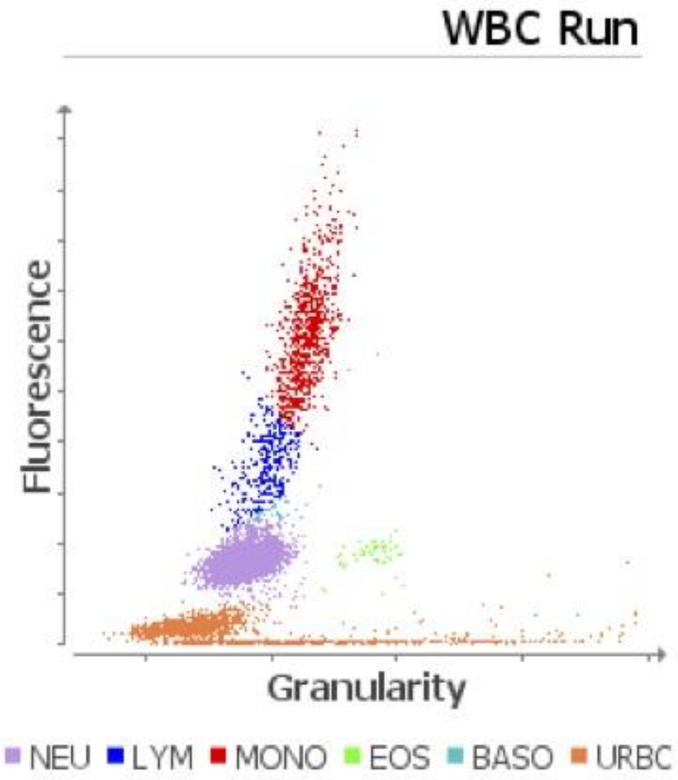
Day 38


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

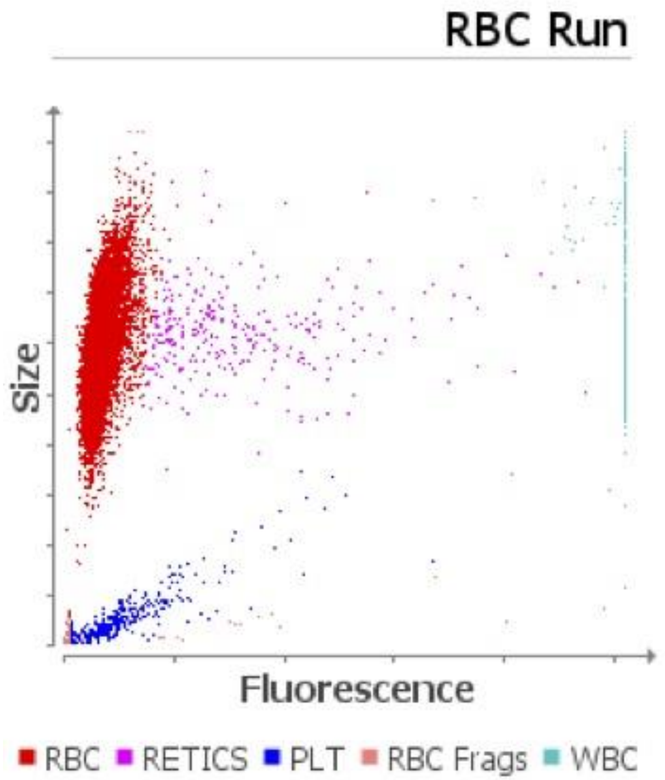

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

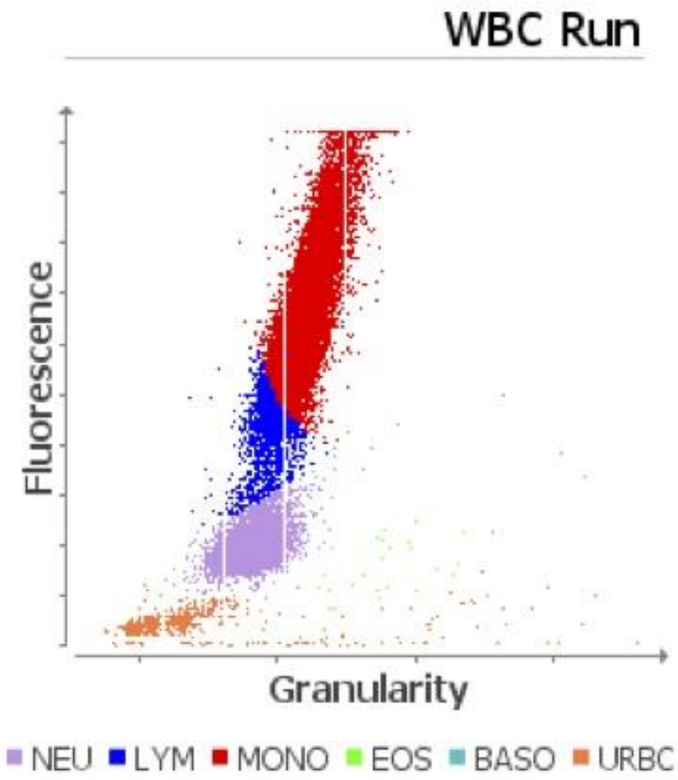
Day 86


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

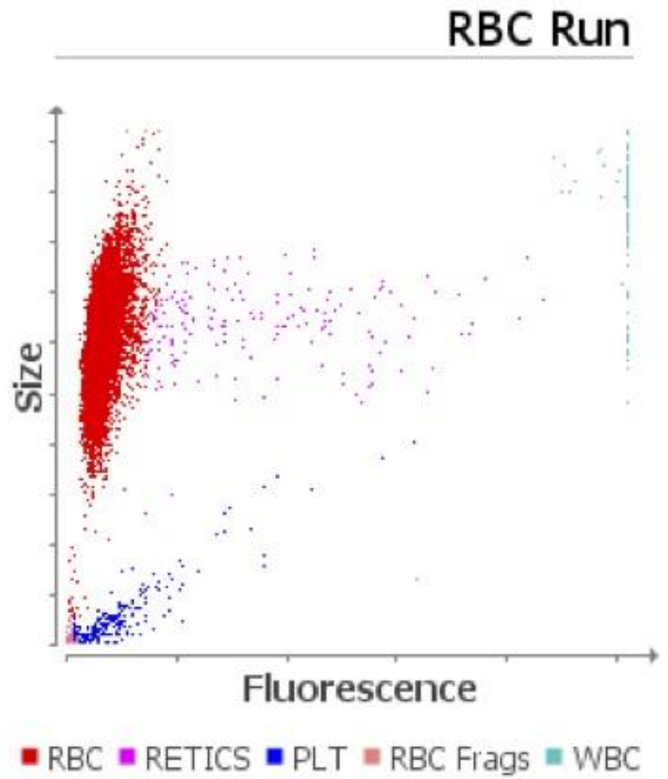

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

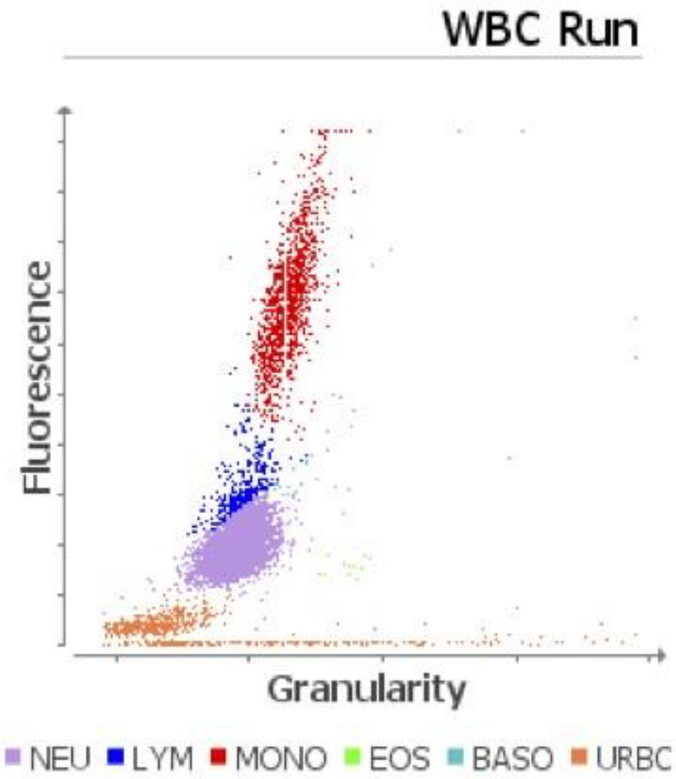
Day 150


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC

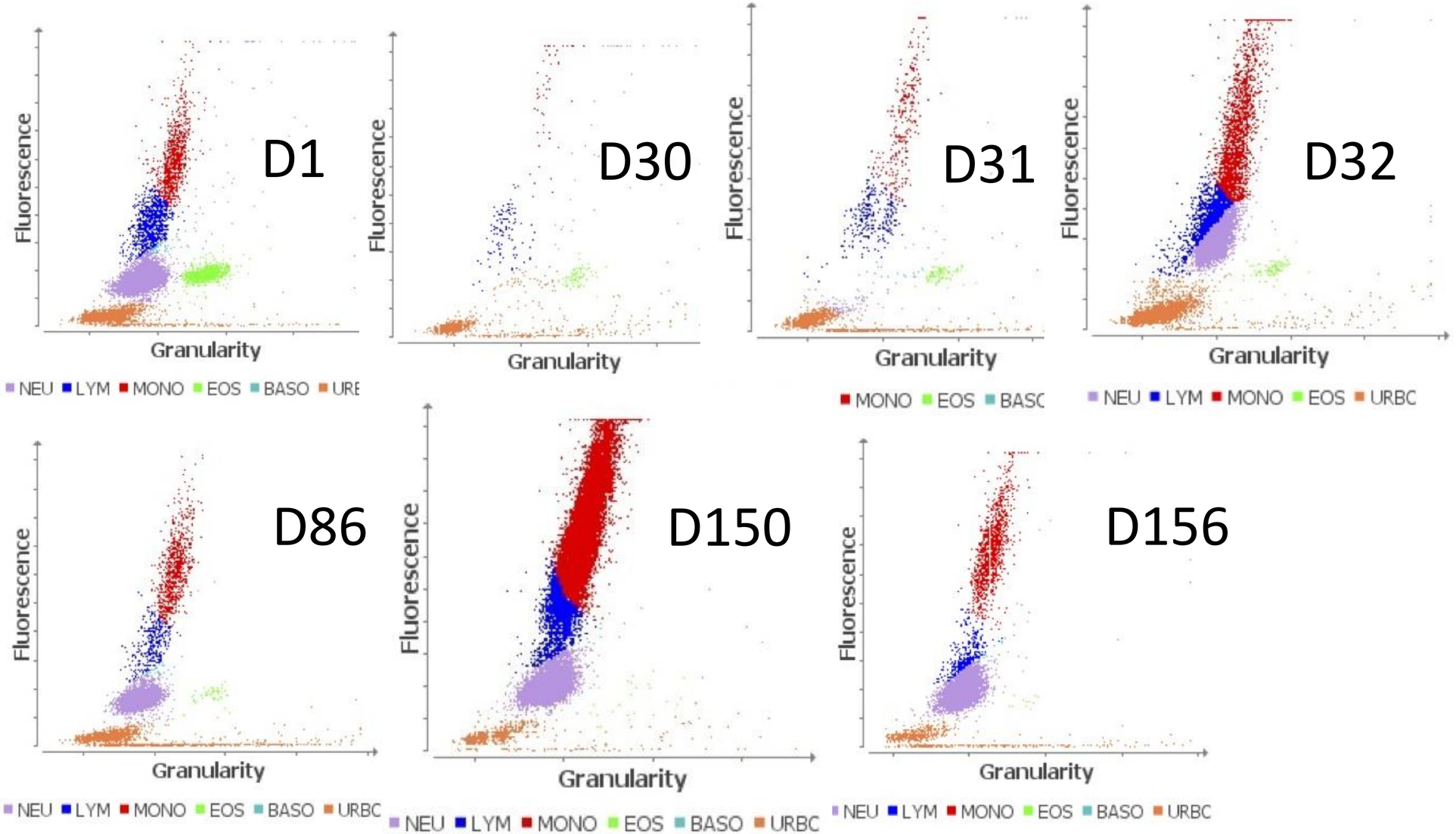

WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

Day 156


RBC Run
Size
Fluorescence
RBC
RETICS
PLT
RBC Frags
WBC


WBC Run
Fluorescence
Granularity
NEU
LYM
MONO
EOS
BASO
URBC

“Pudge”-Sequential WBC



Bone Marrow Recovery