VITICUSGROUP™
WVC ANNUAL CONFERENCE
FEBRUARY 18 - 21, 2024 | LAS VEGAS, NV

Dot Plots, Say What?

Understanding the graphical data of your hematology analyzer

Kim Yore, DVM, MS, DACVIM

Financial Disclosure

I have a direct or indirect relationship with IDEXX, Laboratories Inc. Because of the nature of the relationship, it **will** influence my presentation.

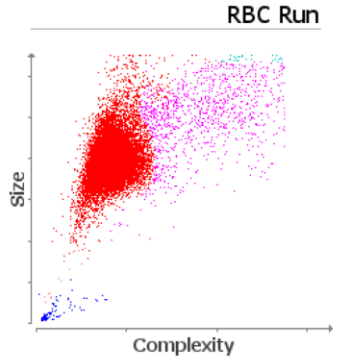
Hematology is more than the numbers on the report

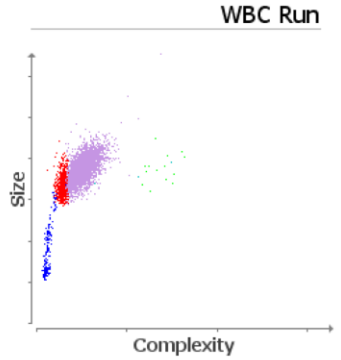
9-year-old, spayed female, mixed breed dog

Referred with suspected immune-mediated thrombocytopenia

Test	Results	Reference Interval	LOW	NORMAL	HIGH
ProCyte One (December 2, 2020 9:48 AM)					
RBC	2.82 M/µL	5.65 - 8.87	LOW		
HCT	19.6 %	37.3 - 61.7	LOW		
HGB	6.4 g/dL	13.1 - 20.5	LOW		
MCV	69.5 fL	61.6 - 73.5			
MCH	22.7 pg	21.2 - 25.9			
MCHC	32.7 g/dL	32.0 - 37.9			
RDW	18.8 %	13.6 - 21.7			
%RETIC	9.6 %				
RETIC	270.3 K/µL	10.0 - 110.0	HIGH		
WBC	* 47.10 K/µL	5.05 - 16.76	HIGH		
%NEU	77.1 %				
%LYM	* 4.2 %				
%MONO	* 18.2 %				
%EOS	0.4 %				
%BASO	0.1 %				
NEU	36.33 K/µL	2.95 - 11.64	HIGH		
LYM	* 2.00 K/µL	1.05 - 5.10			
MONO	* 8.56 K/µL	0.16 - 1.12	HIGH		
EOS	0.18 K/µL	0.06 - 1.23			
BASO	0.04 K/µL	0.00 - 0.10			
PLT	16 K/µL	148 - 484	LOW		
MPV	8.1 fL	8.7 - 13.2	LOW		
PDW	--.-- fL	9.1 - 19.4			
PCT	--.-- %	0.14 - 0.46			

* Confirm with dot plot and/or blood film review.

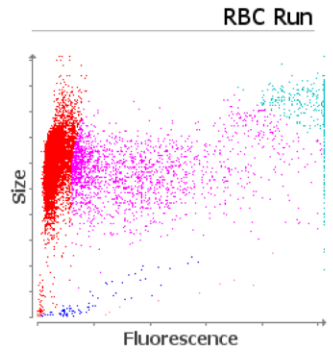


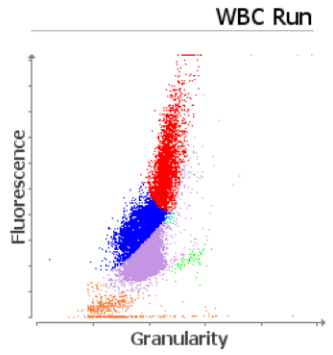


1. Anemia with reticulocytosis - Likely regenerative anemia.

Test	Results	Reference Interval	LOW	NORMAL	HIGH
ProCyte Dx (December 2, 2020 9:51 AM)					
RBC	2.70 M/µL	5.65 - 8.87	LOW		
HCT	19.4 %	37.3 - 61.7	LOW		
HGB	6.6 g/dL	13.1 - 20.5	LOW		
MCV	71.9 fL	61.6 - 73.5			
MCH	24.4 pg	21.2 - 25.9			
MCHC	34.0 g/dL	32.0 - 37.9			
RDW	16.7 %	13.6 - 21.7			
%RETIC	8.7 %				
RETIC	235.4 K/µL	10.0 - 110.0	HIGH		
RETIC-HGB	23.4 pg	22.3 - 29.6			
WBC	46.75 K/µL	5.05 - 16.76	HIGH		
%NEU	* 64.4 %				
%LYM	* 22.7 %				
%MONO	* 12.6 %				
%EOS	0.2 %				
%BASO	0.1 %				
NEU	* 30.10 K/µL	2.95 - 11.64	HIGH		
BAND	* Suspected				
LYM	* 10.61 K/µL	1.05 - 5.10	HIGH		
MONO	* 5.90 K/µL	0.16 - 1.12	HIGH		
EOS	0.10 K/µL	0.06 - 1.23			
BASO	0.04 K/µL	0.00 - 0.10			
PLT	* 1 K/µL	148 - 484	LOW		
MPV	14.8 fL	8.7 - 13.2	HIGH		
PDW	0.0 fL	9.1 - 19.4	LOW		
PCT	--.-- %	0.14 - 0.46			

* Confirm with dot plot and/or blood film review.



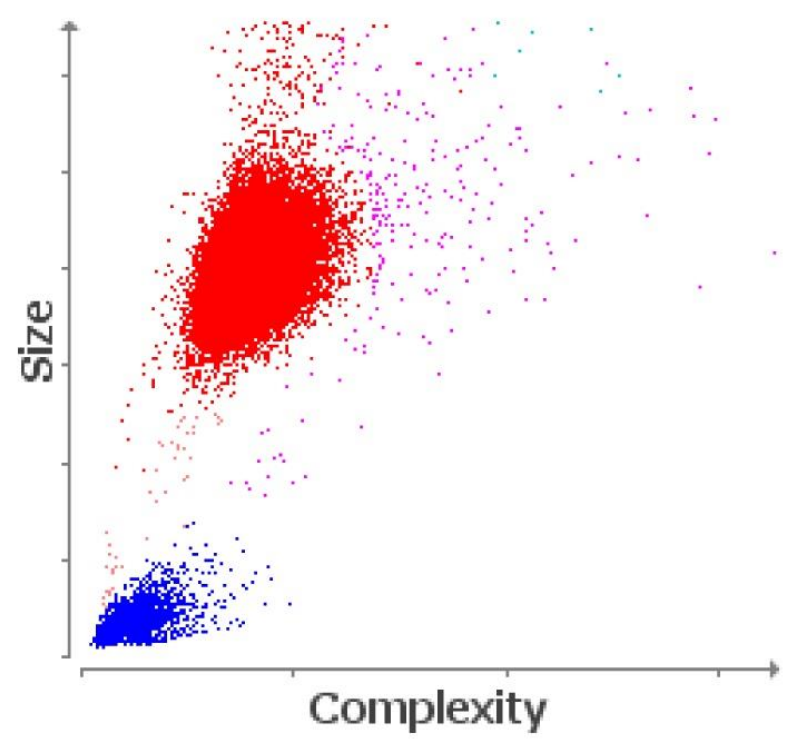


1. Anemia with reticulocytosis - Likely regenerative anemia.

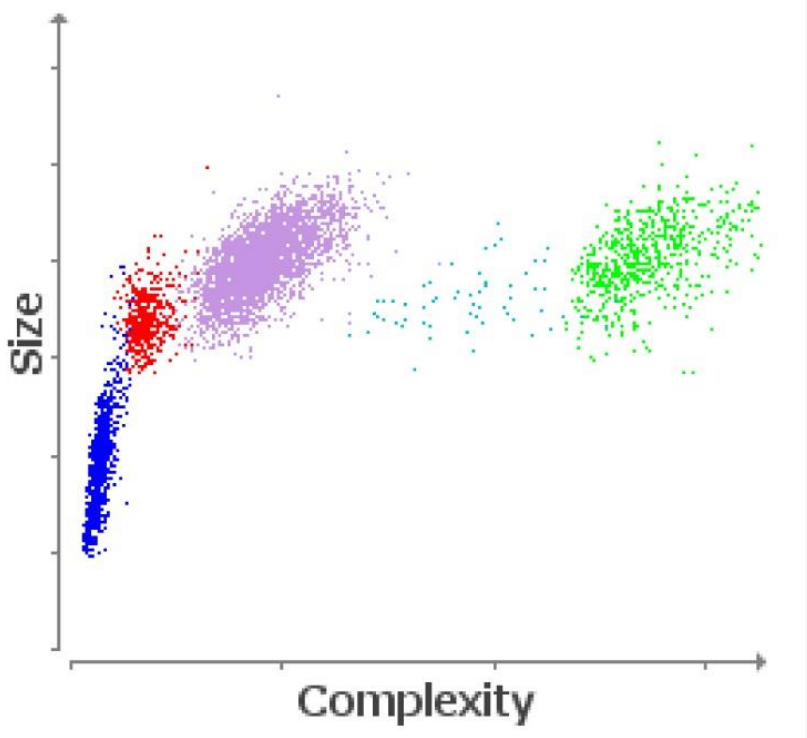
1. Immature and/or toxic neutrophils likely present - Consider inflammation.

What are dot plots?

Red Blood Cell Run (ProCyte One®)

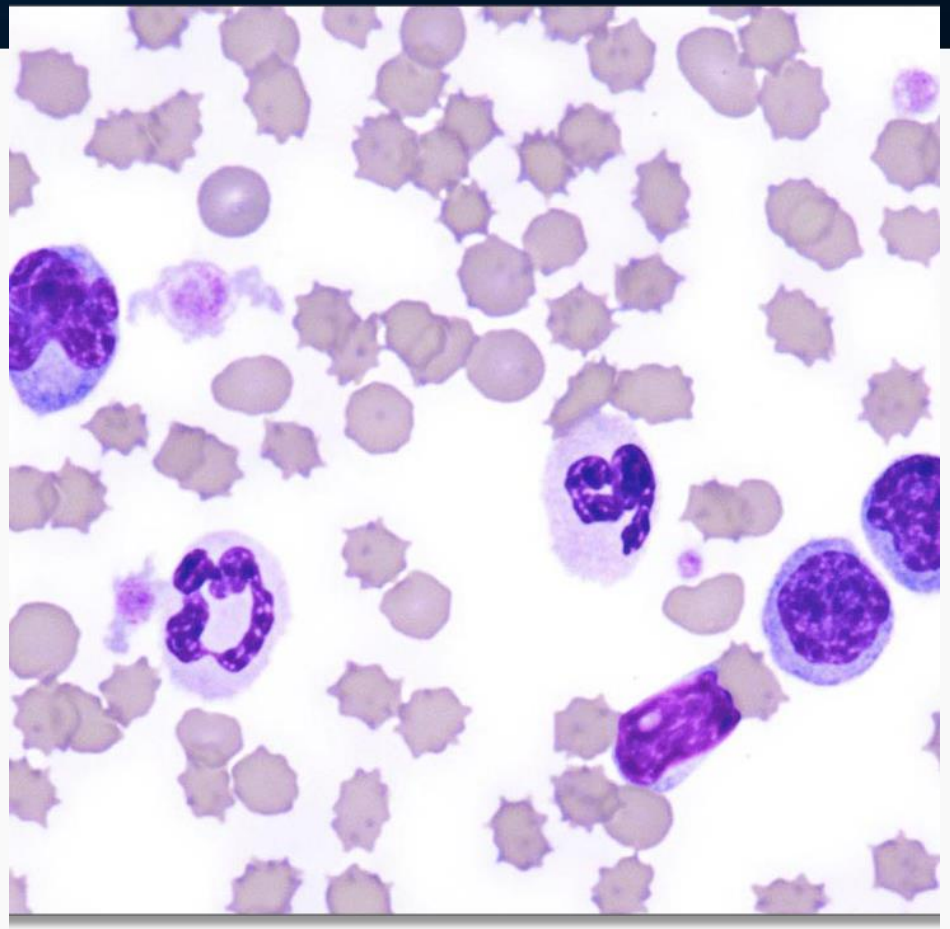


White Blood Cell Run (ProCyte One®)



Canine

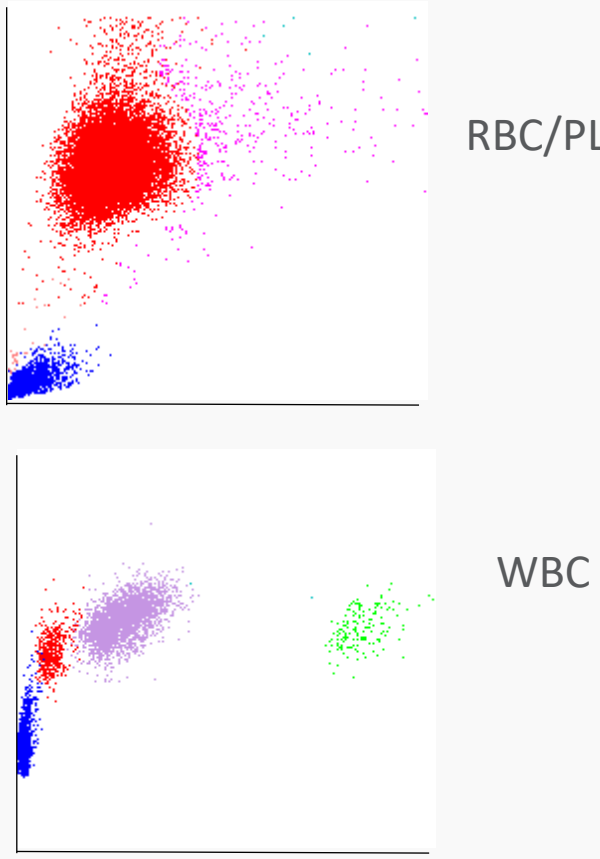
What are Dot Plots?



Dot Plots, the graphical data from laser flow cytometry CBC analyzers

- Assist with confirming the numerical values of the CBC
- Provide insight into blood morphology and direct assessment of blood morphology

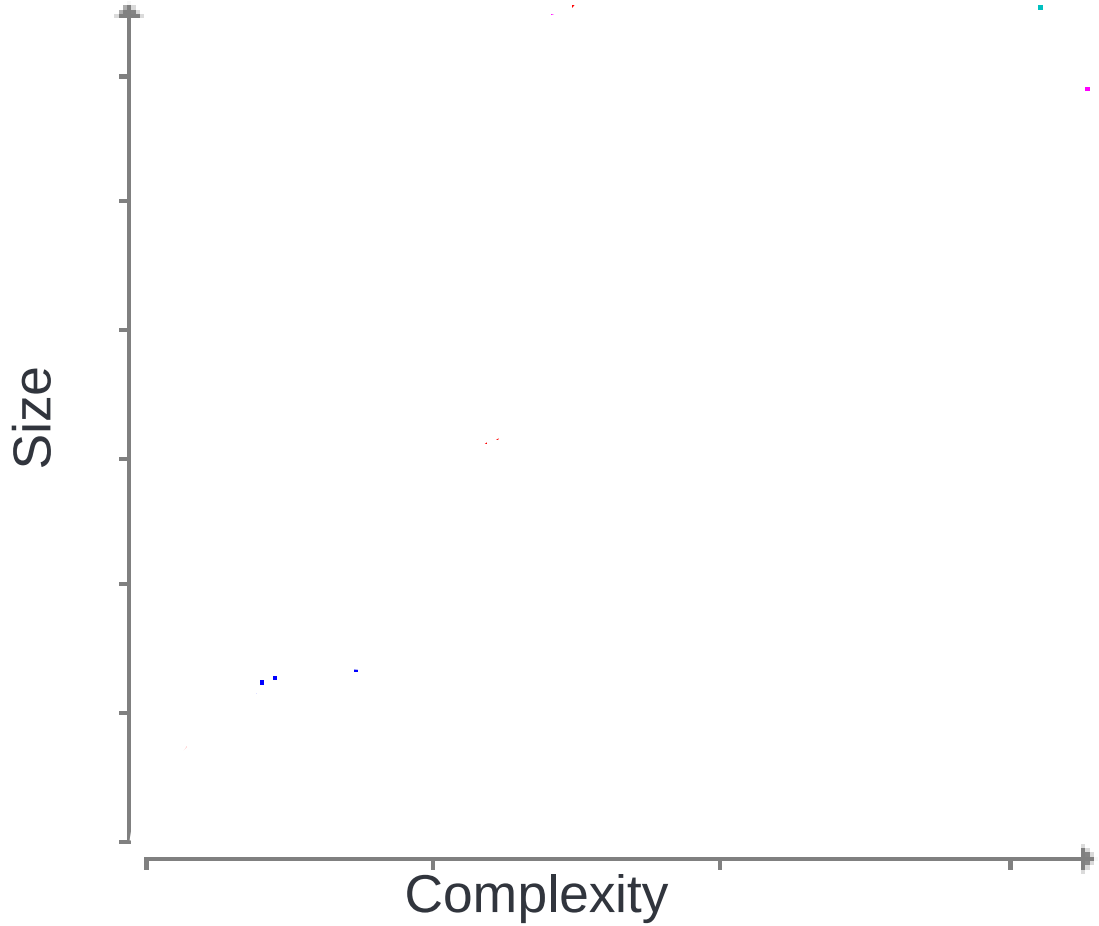
What are Dot Plots?



Visual Representation of the CBC

- Two types
  - Red blood cell/platelet (RBC/PLT) dot plots
  - White blood cell (WBC) dot plots
- X Axis
  - Mostly information about cell complexity
- Y Axis
  - Mostly information about cell size

Plotting RBCs and platelets

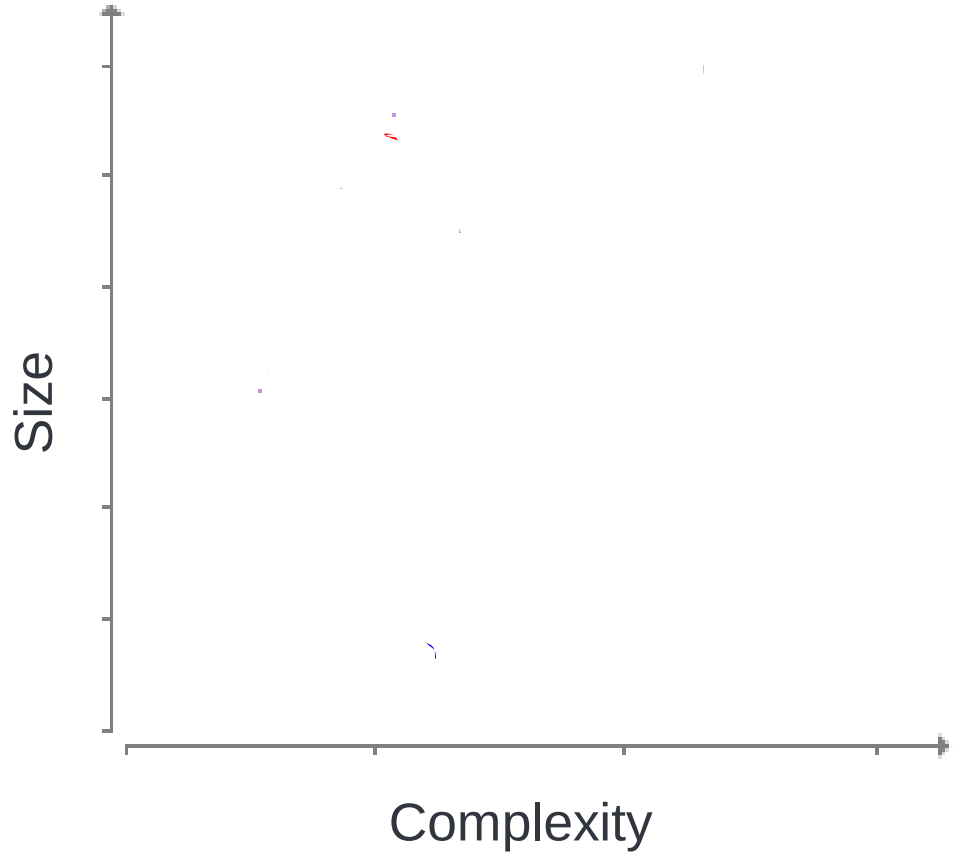


Red blood cells

Reticulocytes

Platelets

WBC dot plot



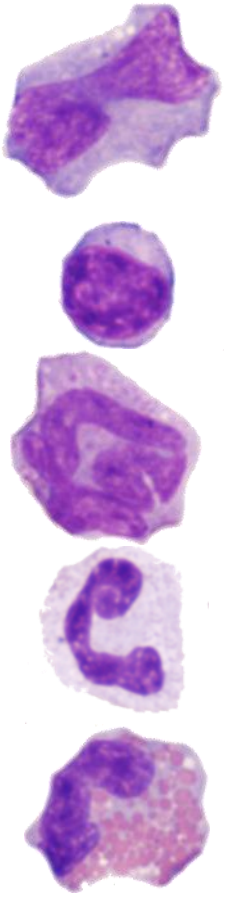
Monocyte

Lymphocyte

Basophil

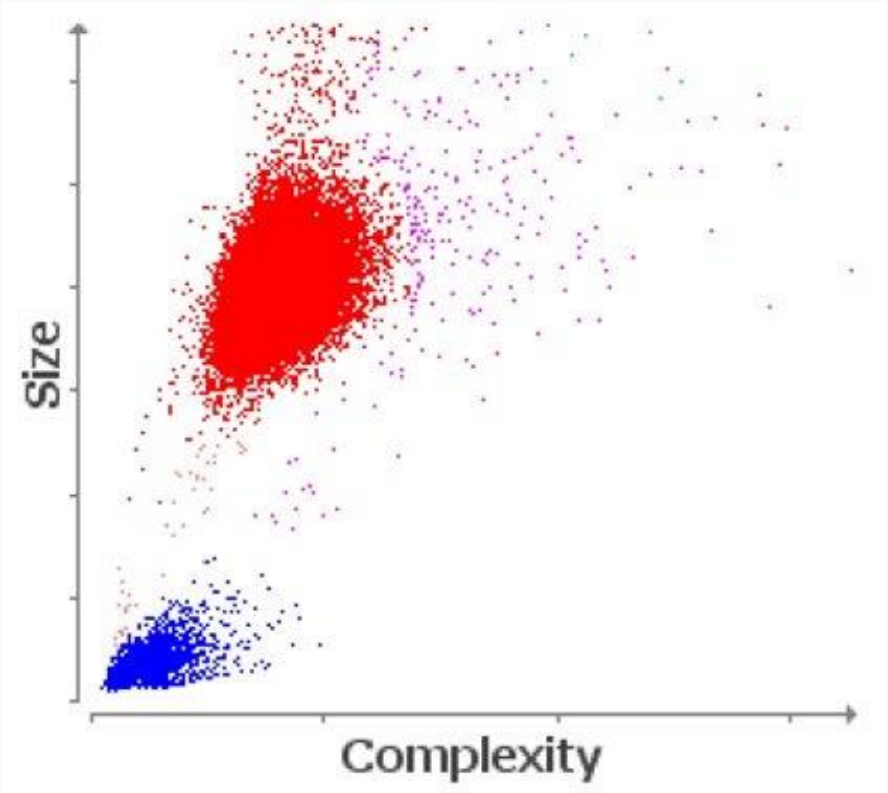
Neutrophil

Eosinophil

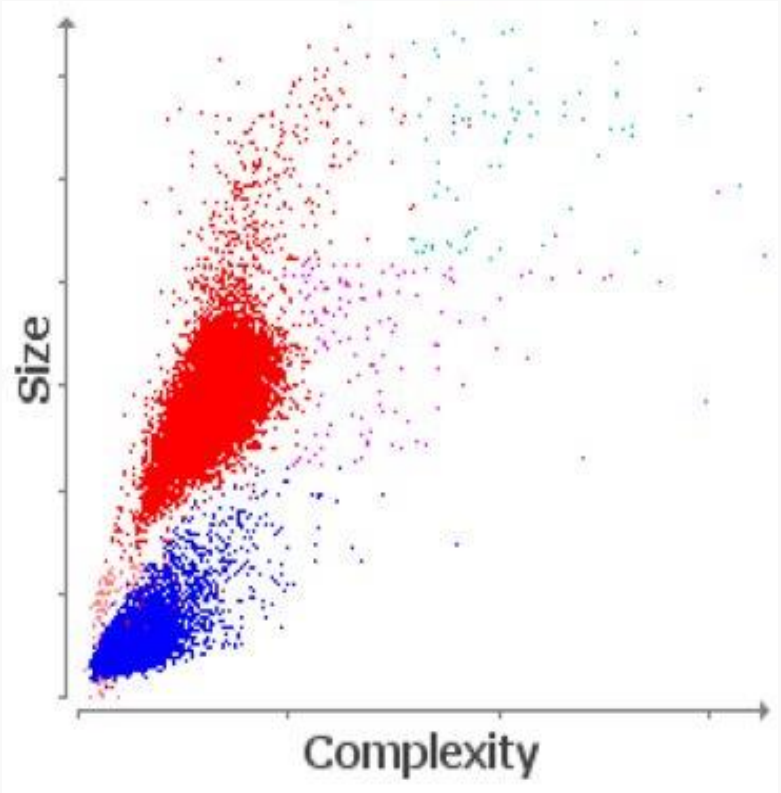


Normal Canine and Feline RBC/PLT Dot Plot

Canine



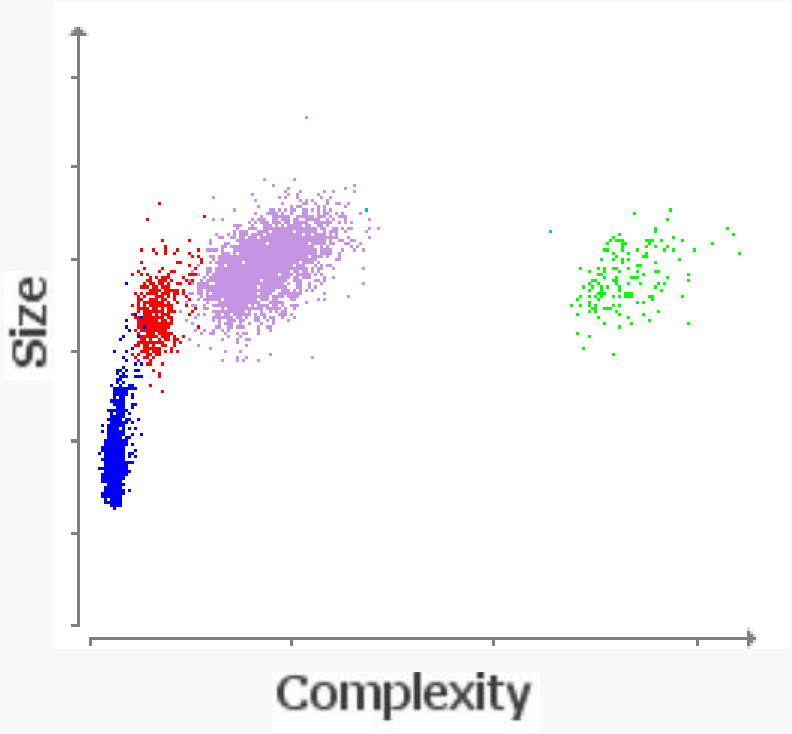
Feline



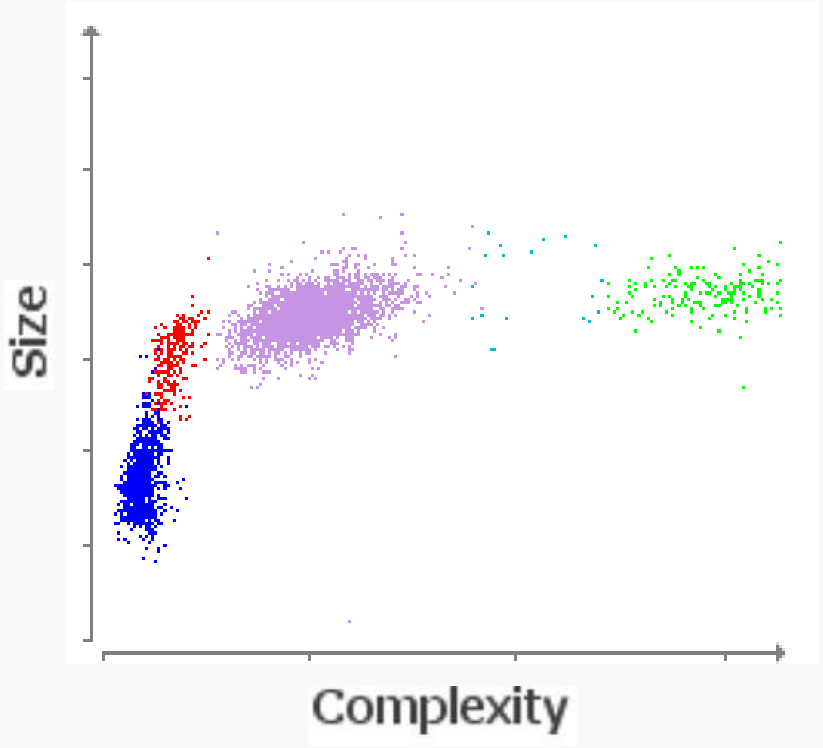
RBC RETICS PLT RBC Frags WBC

Normal Canine and Feline WBC Dot Plot

Canine



Feline



NEU LYM MONO EOS BASO

Chunk: 7-year-old, FS, Siamese Cat

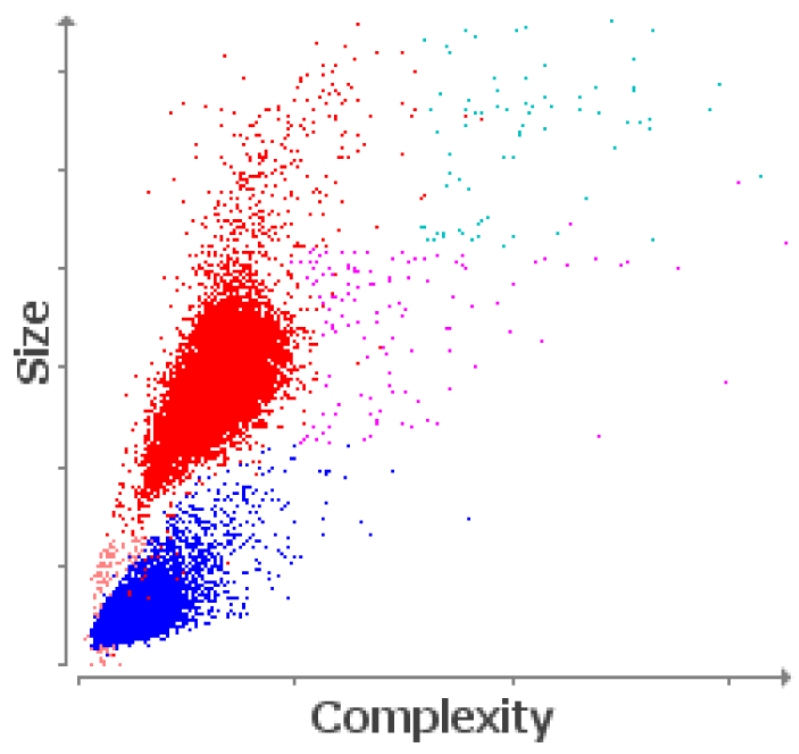
- Presented for several days of inappetence
- Primary clinical presentation
  - Jaundice
  - Fever
  - Pale mucous membranes

Photo credit: Kim Yore, Westbrook, Maine

RBC/PLT Dot Plot

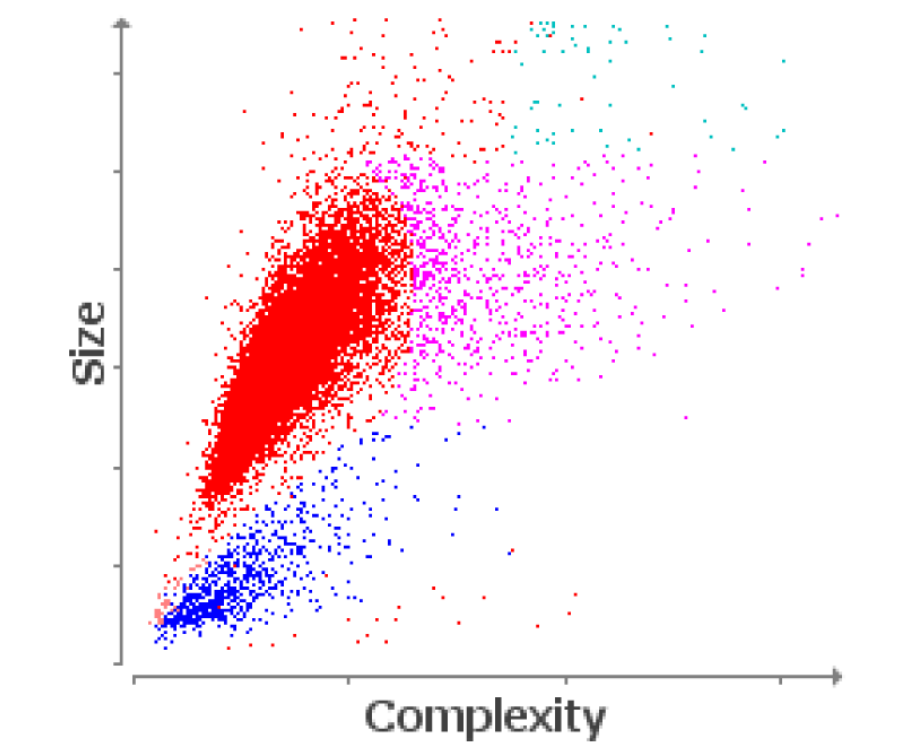
Normal Feline

Chunk



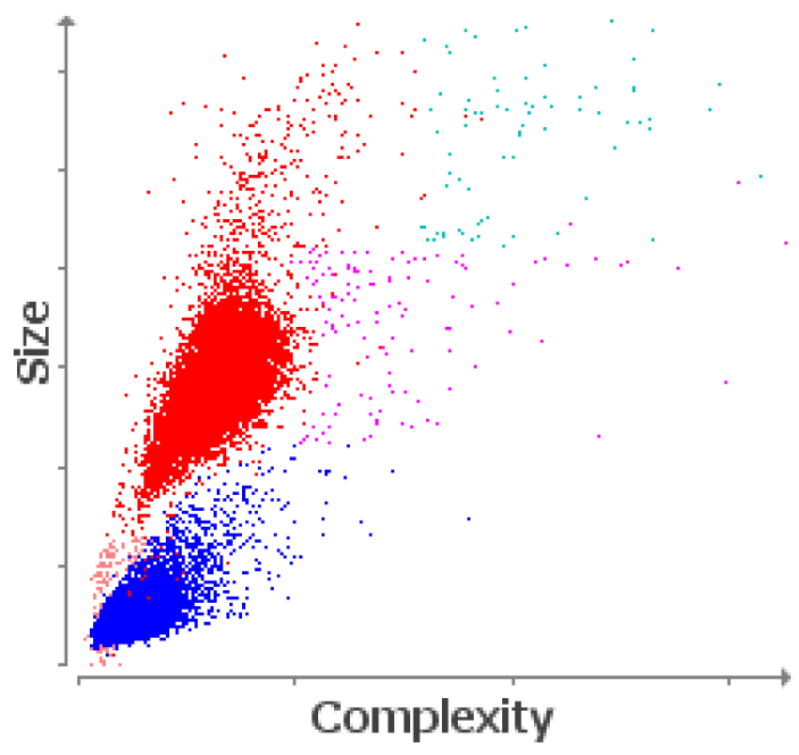
Is this a normal or abnormal dot plot?

What is abnormal about it?



Assessment of the Abnormal RBC/PLT Dot Plot

Normal Feline



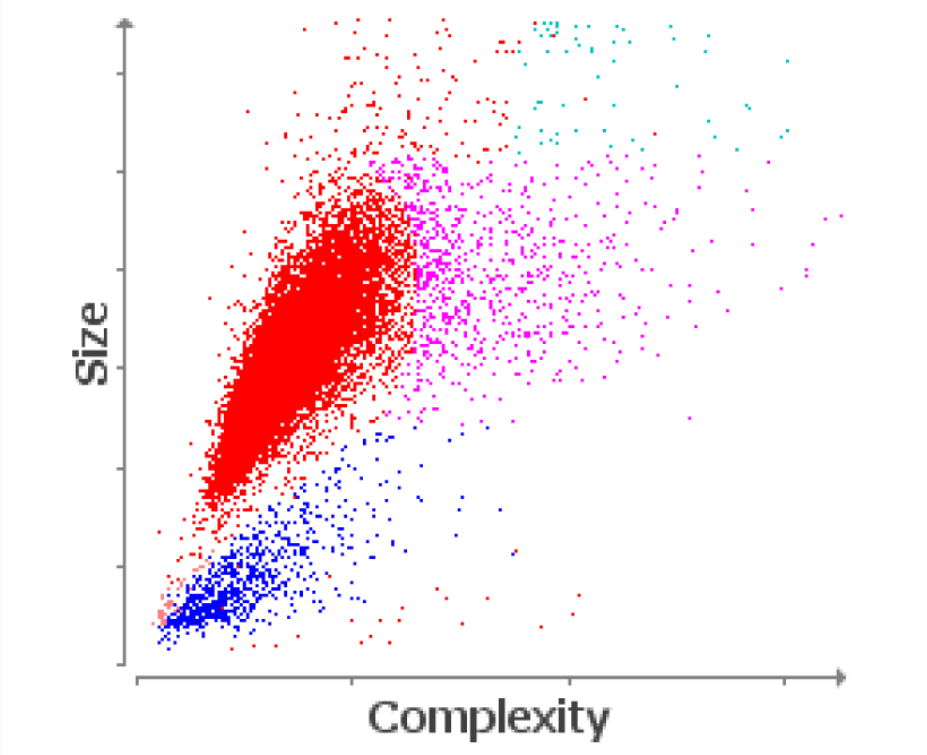
Is there good separation of the cell populations?

If YES, confidence in the automated numbers on the CBC

If NO, likely that biological morphologic changes are present

- Interpret numeric data with caution

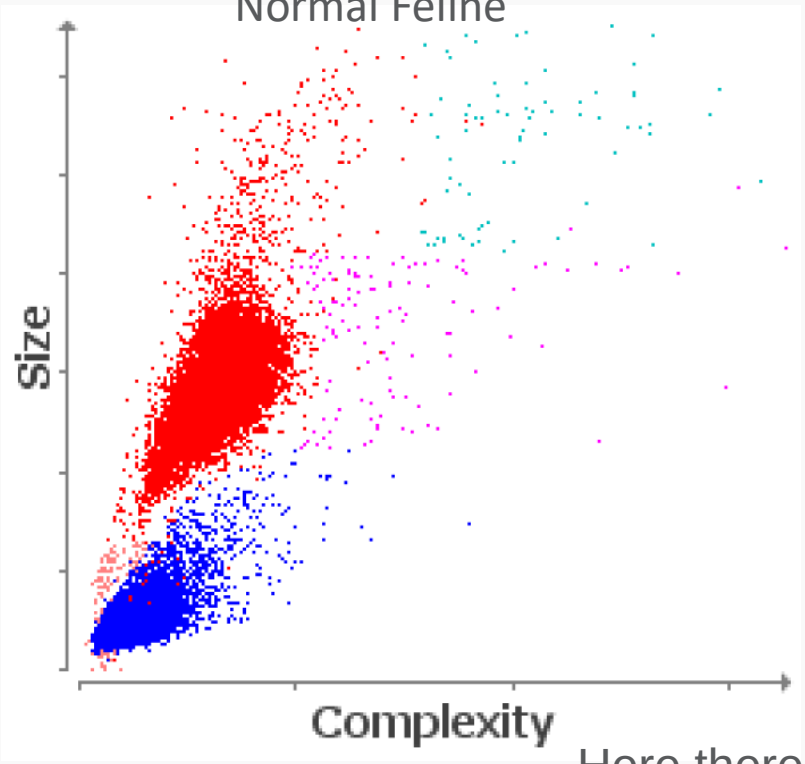
Chunk



Here there is good separation of the cell populations.

Assessment of the Abnormal RBC/PLT Dot Plot

Normal Feline

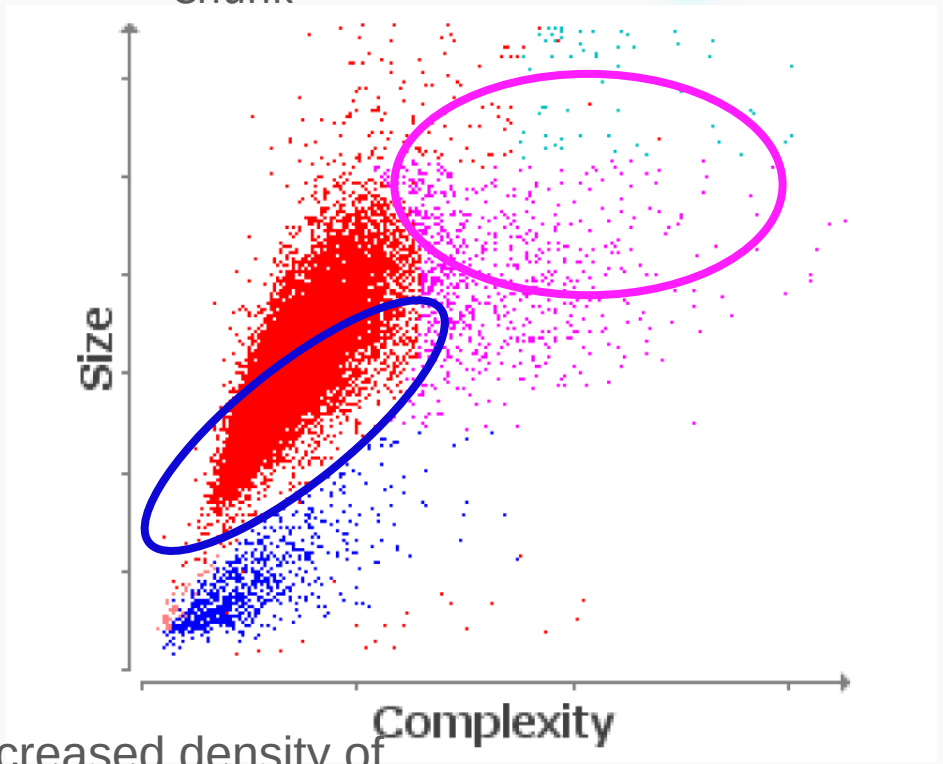


Are there changes in the densities of the cell clusters?

One can visually determine changes in quantity of

- reticulocytes
- platelets

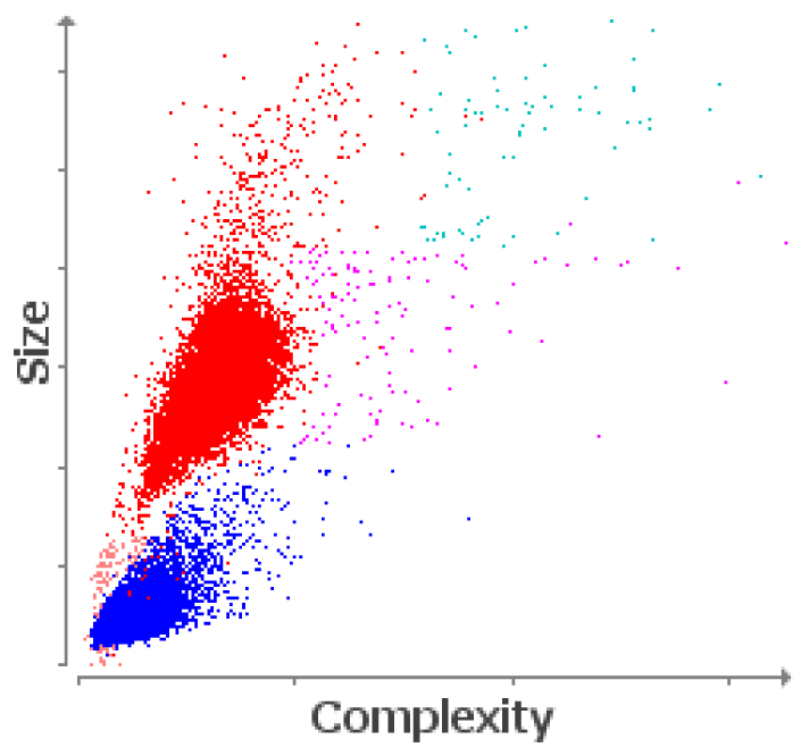
Chunk



Here there is indication of reticulocytosis (increased density of dots in the pink circle) and thrombocytopenia (decreased density of dots in the blue circle)

Assessment of the Abnormal RBC/PLT Dot Plot

Normal Feline

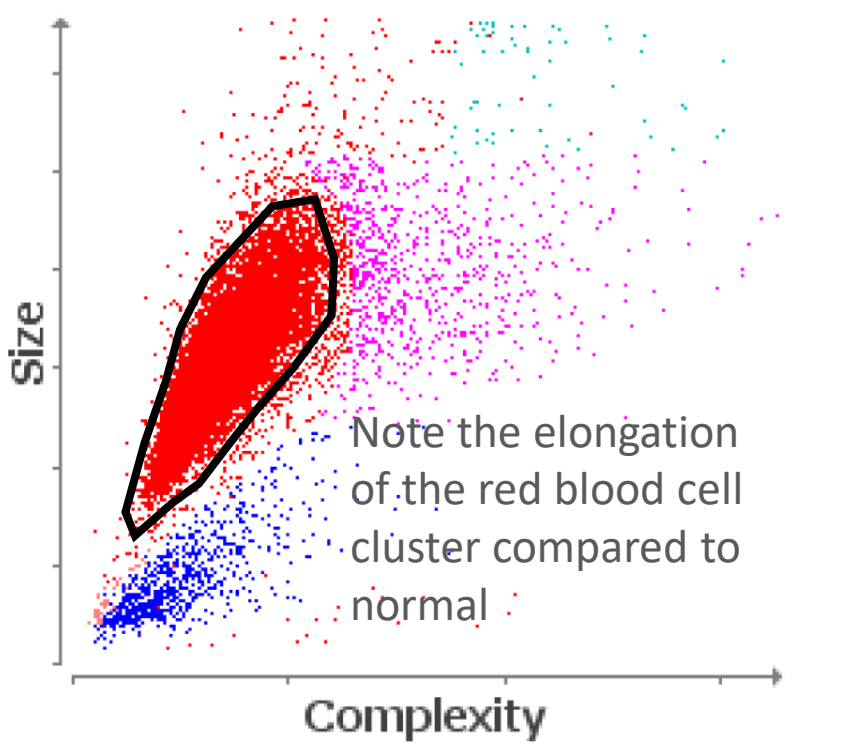


Are there changes in the location of the cells?

Biologic changes in cell morphology result in change in **the shape and location** of the cell clusters compared to normal

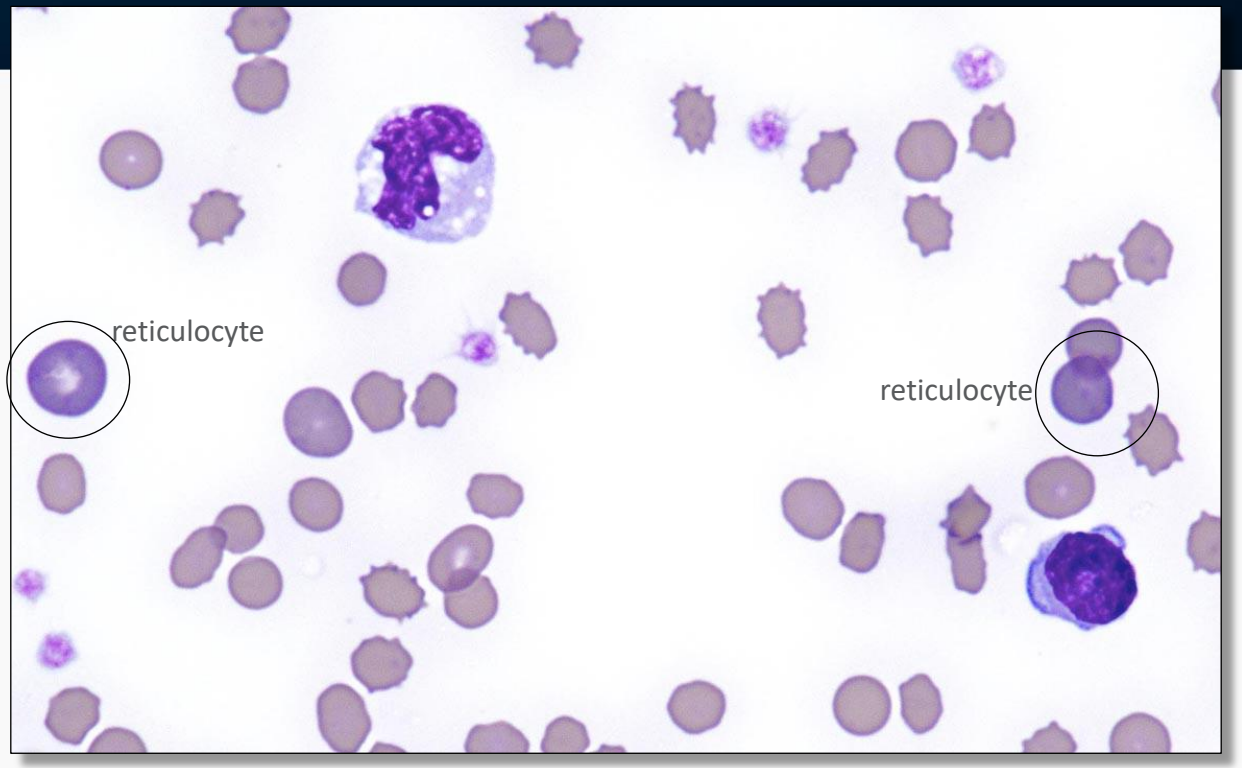
Directs a targeted assessment of blood morphology

Chunk



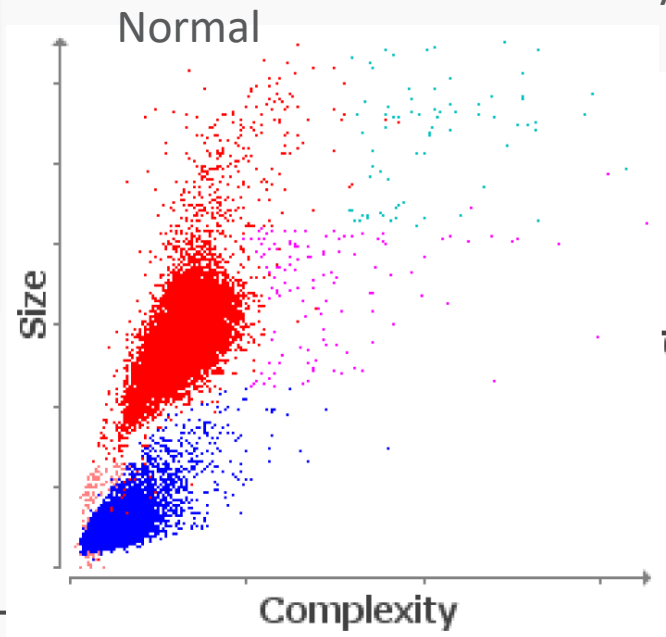
Abnormal positioning of digitized events supports abnormal cell morphology – ***blood morphology must be evaluated***

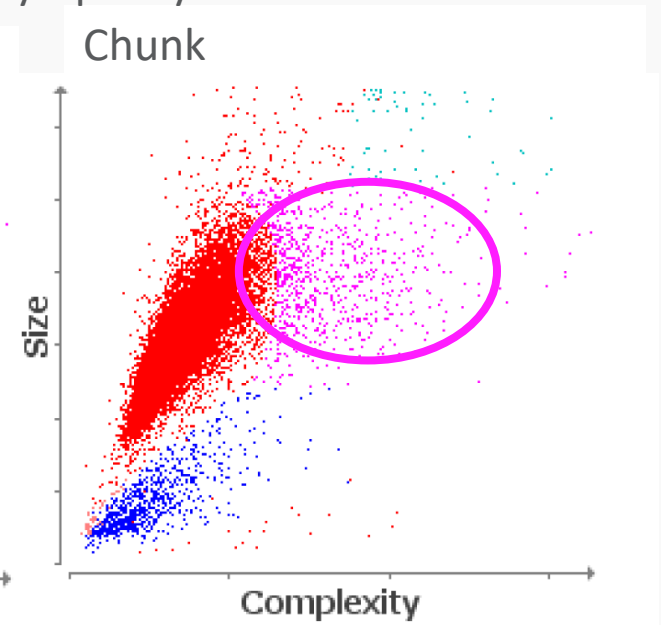
Perform a targeted assessment of blood morphology



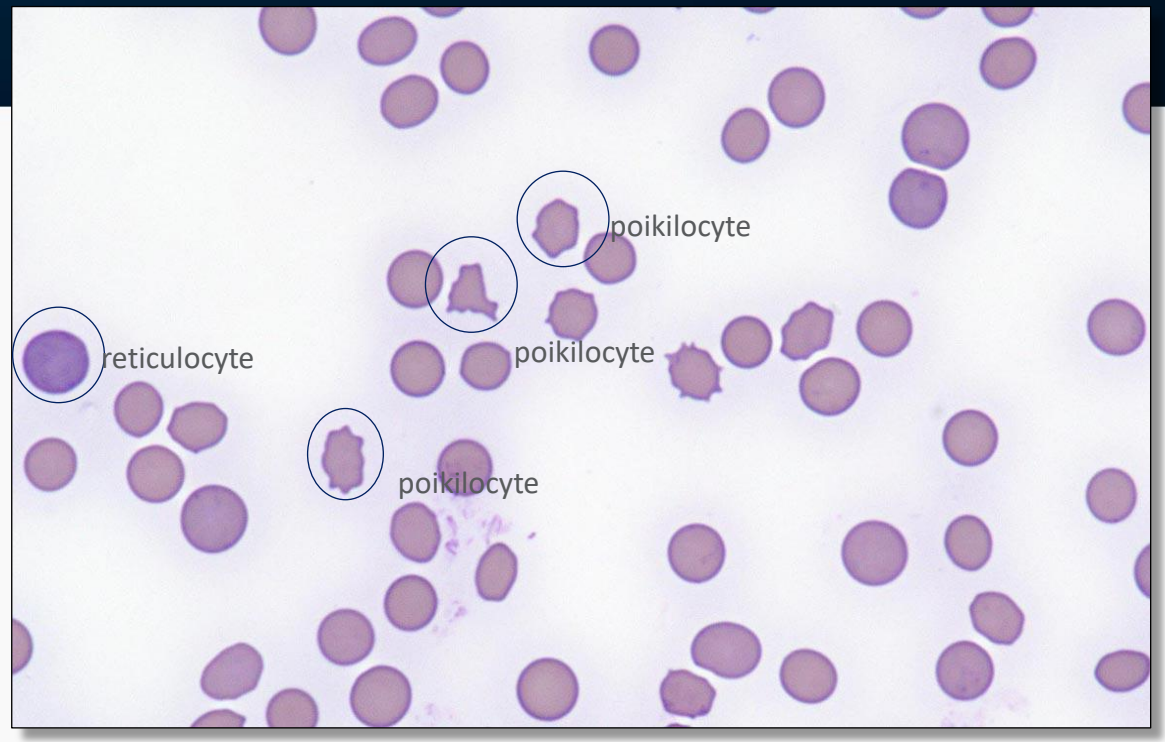
Decreased RBC density
Moderate polychromasia

Moderate poikilocytosis
Monocyte
Intermediate sized lymphocyte



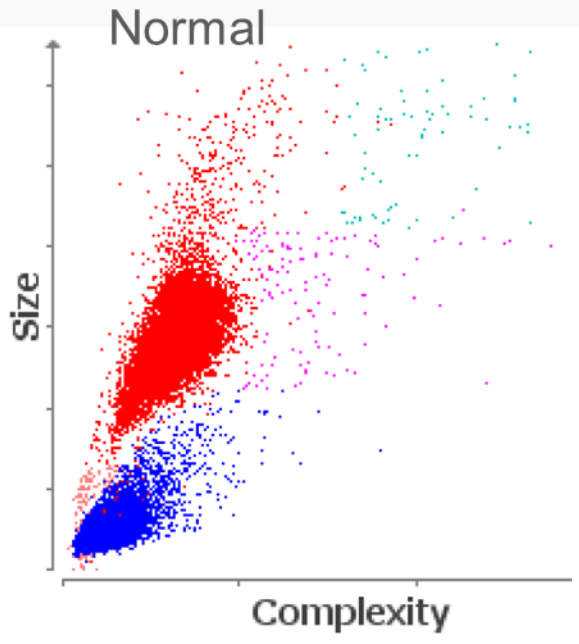


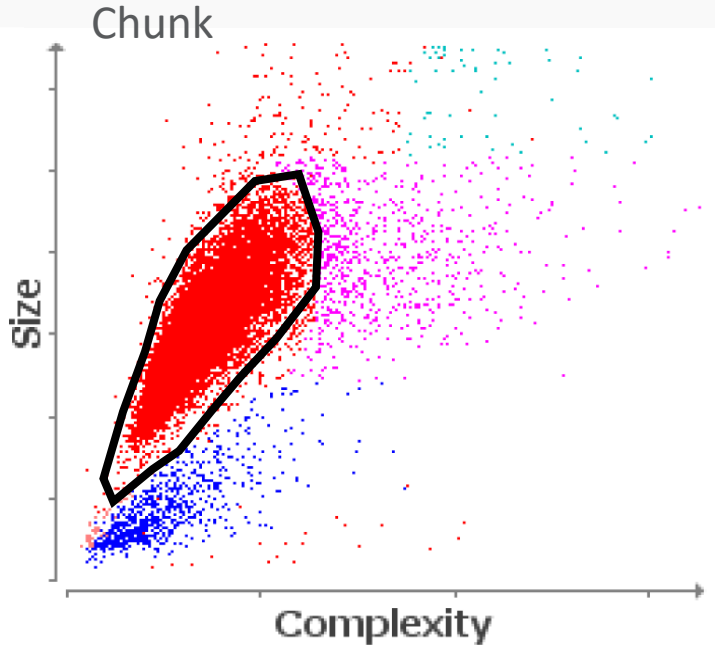
Perform a targeted assessment of blood morphology



Decreased RBC density
Moderate polychromasia

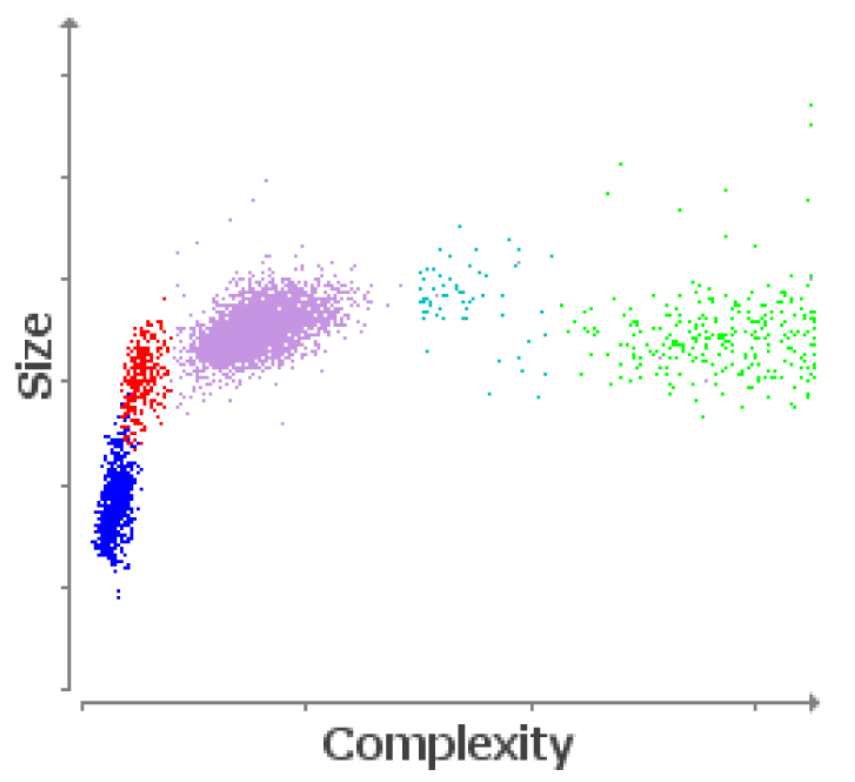
Moderate poikilocytosis





WBC Dot Plot

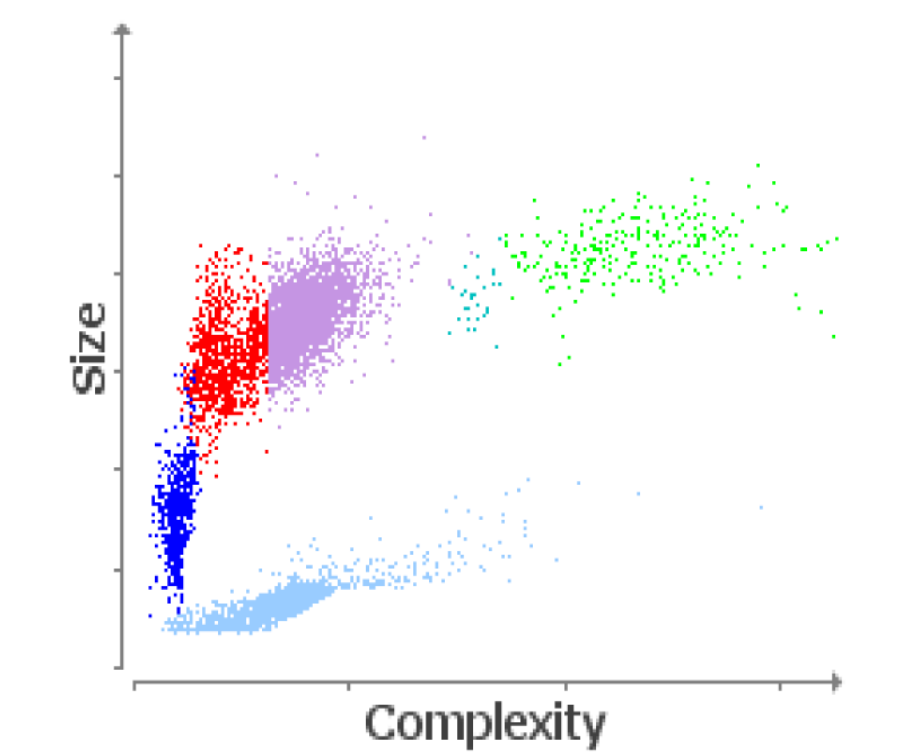
Normal



Is this a normal or abnormal dot plot?

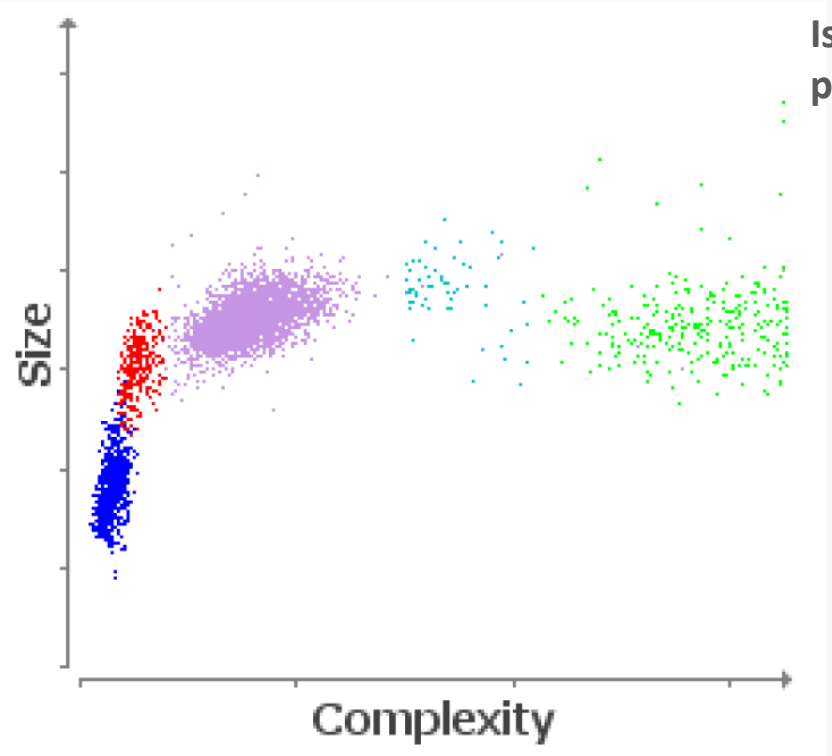
What is abnormal about it?

Chunk



WBC Dot Plot

Normal



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If YES, confidence in the automated numbers on the CBC

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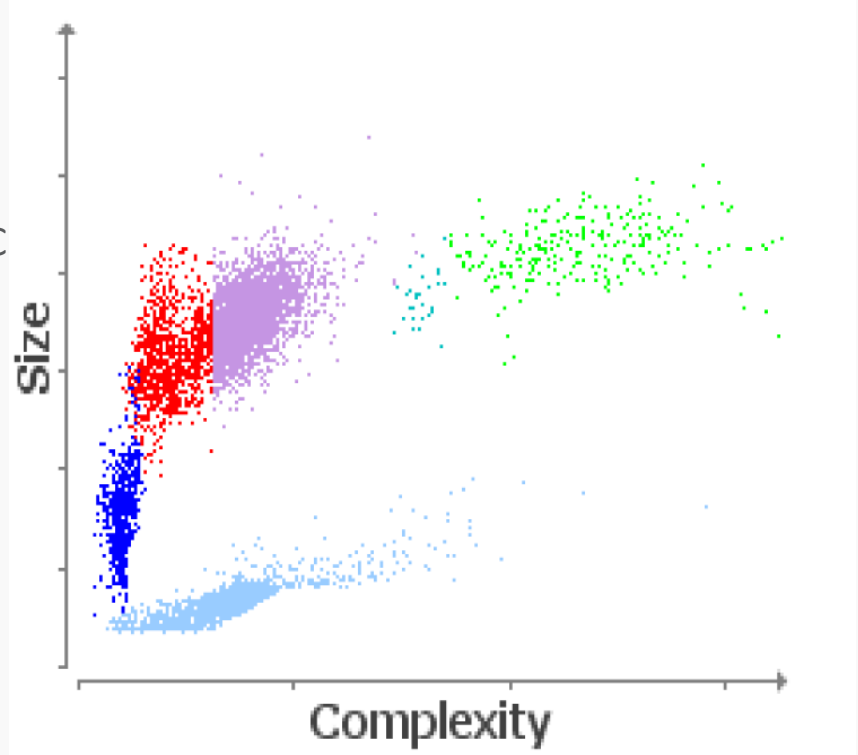
Chunk



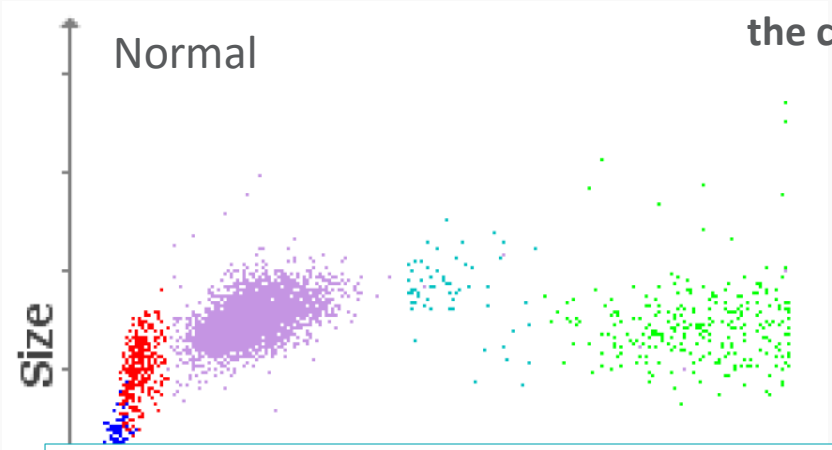
Here there is NOT good separation of the cell populations.

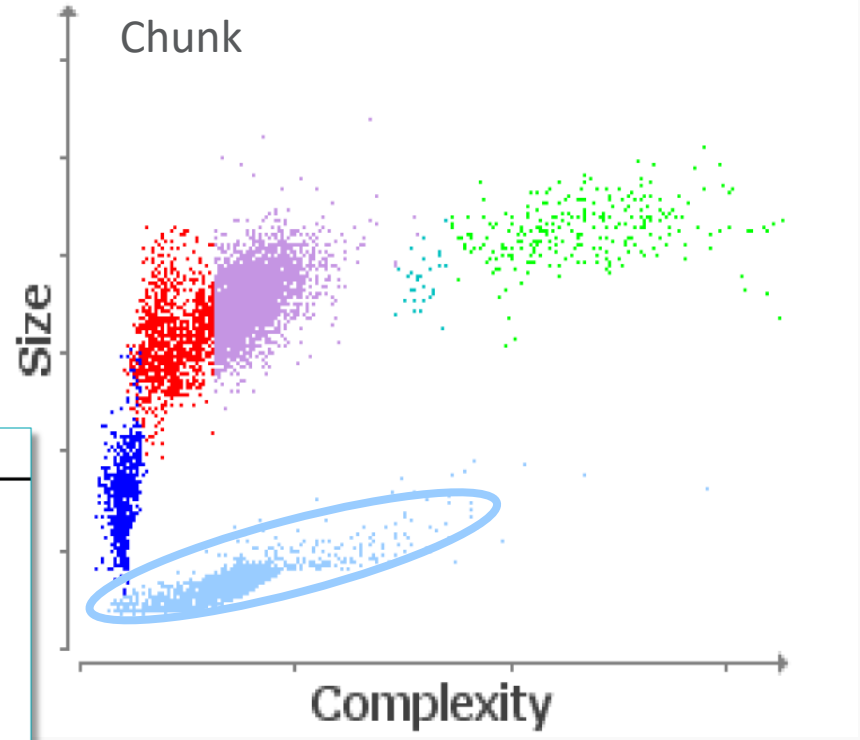
WBC Dot Plot

Are there changes in the densities of the cell clusters?

One can visually determine changes in quantity of

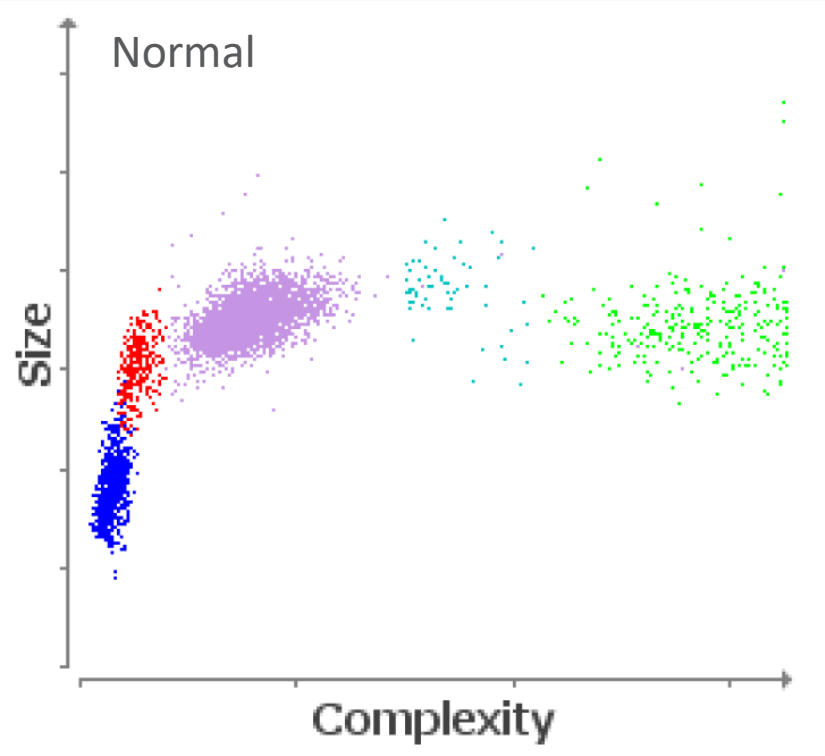
- The different WBCs
- Presence of platelet clumps





Test	Results	Reference Interval	LOW	NORMAL	HIGH
ProCyte One (October 6, 2021 3:45 PM)					
WBC	25.33 x10^9/L	2.87 - 17.02	HIGH		
NEU	11.42 x10^9/L	2.30 - 10.29	HIGH		
LYM	0.78 x10^9/L	0.92 - 6.88	LOW		
MONO	* 12.77 x10^9/L	0.05 - 0.67	HIGH		
EOS	0.32 x10^9/L	0.17 - 1.57			
BASO	0.03 x10^9/L	0.01 - 0.26			

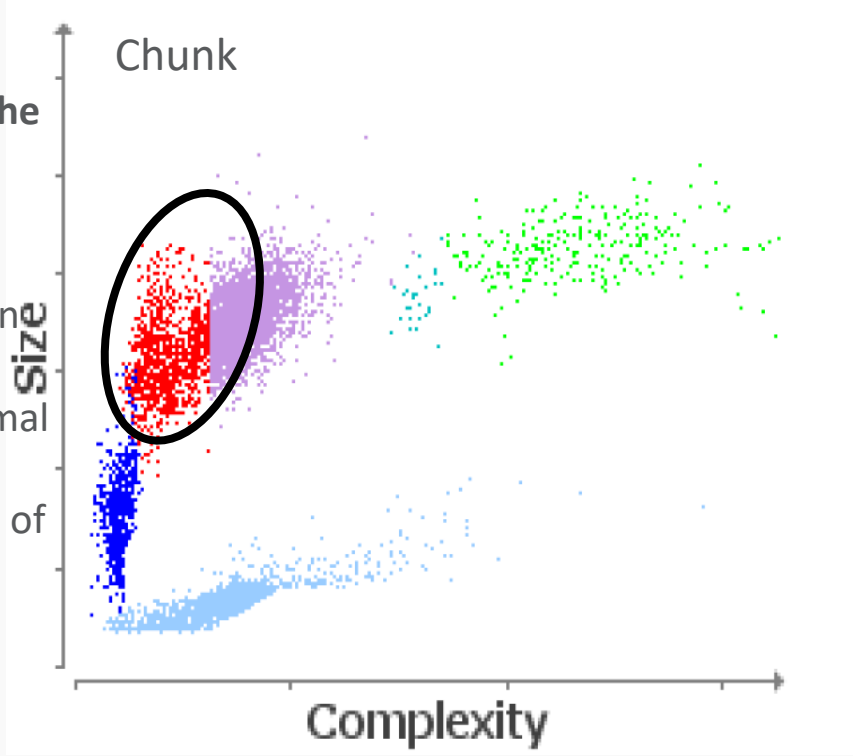
WBC Dot Plot



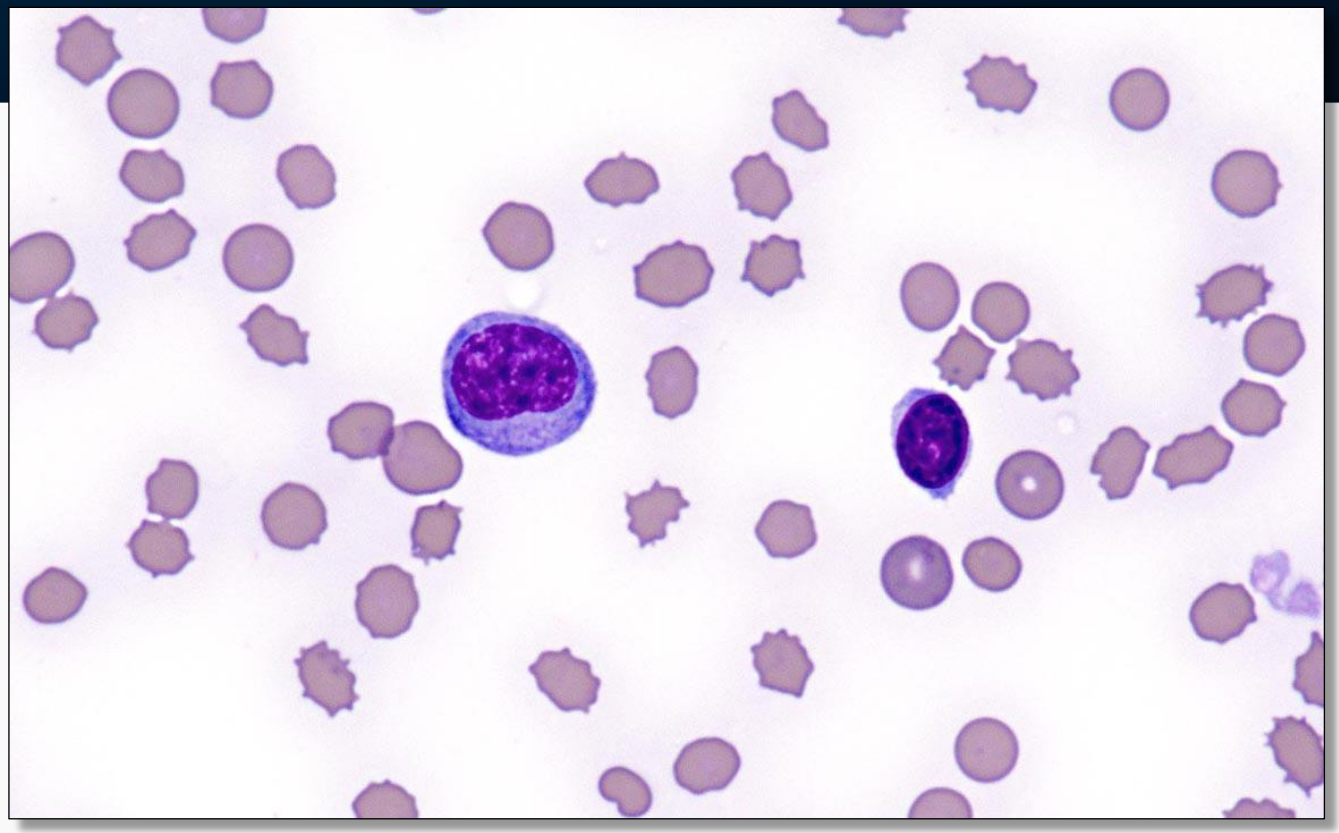
Are there changes in the location the cells?

Biologic change in cell morphology result in change in **the shape and location** of the cell clusters compared to normal

Directs a targeted assessment of blood morphology

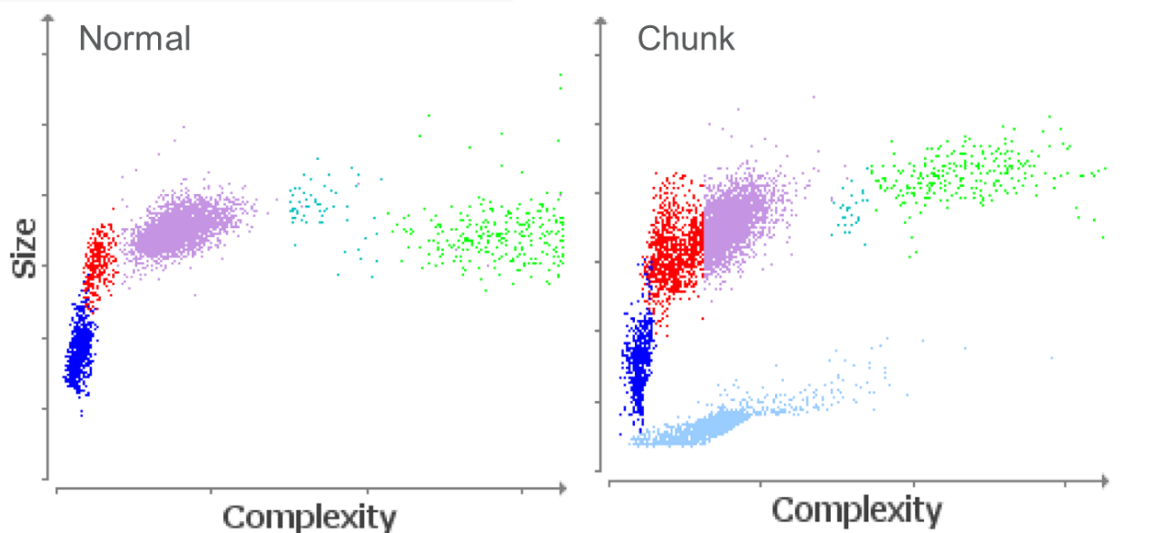


Perform a targeted assessment of blood morphology

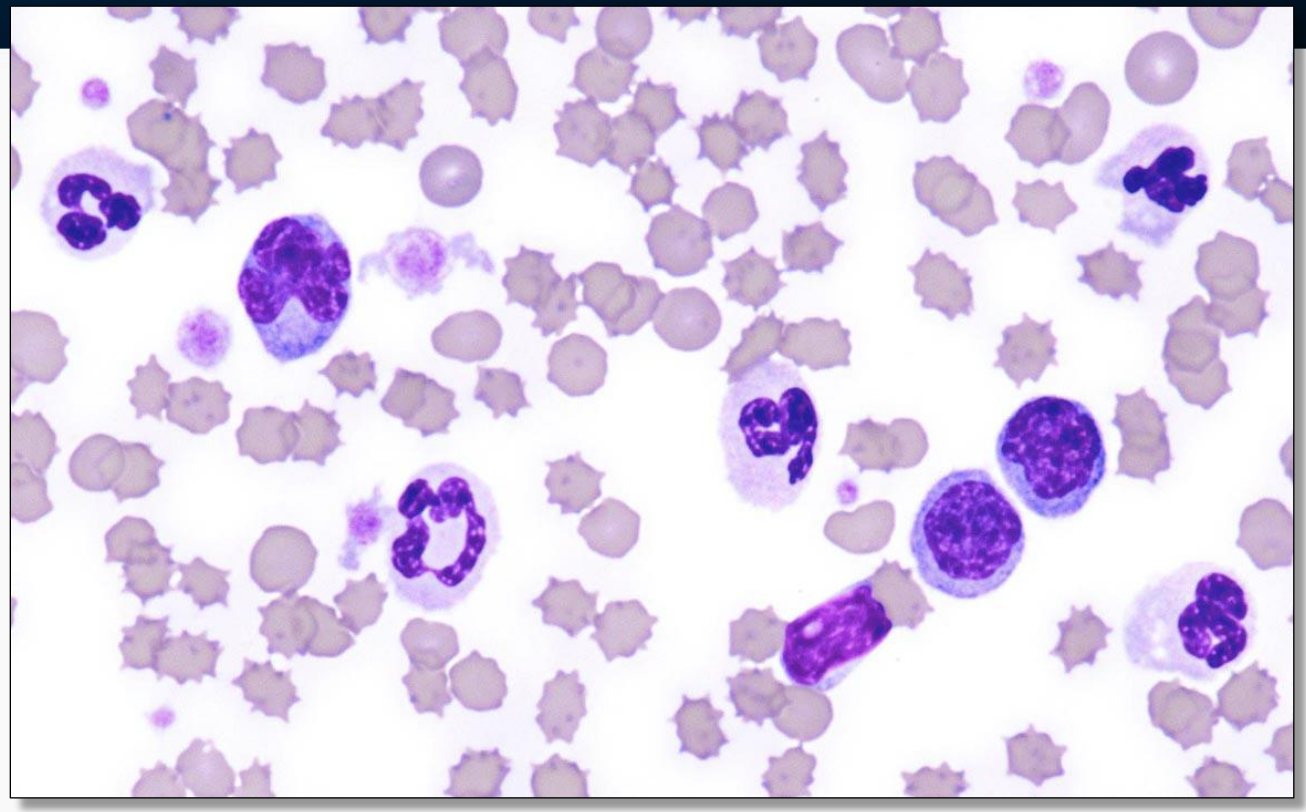


Decreased RBC density
Moderate polychromasia
Moderate poikilocytosis

Normal small lymphocyte
Large and immature appearing mononuclear cell

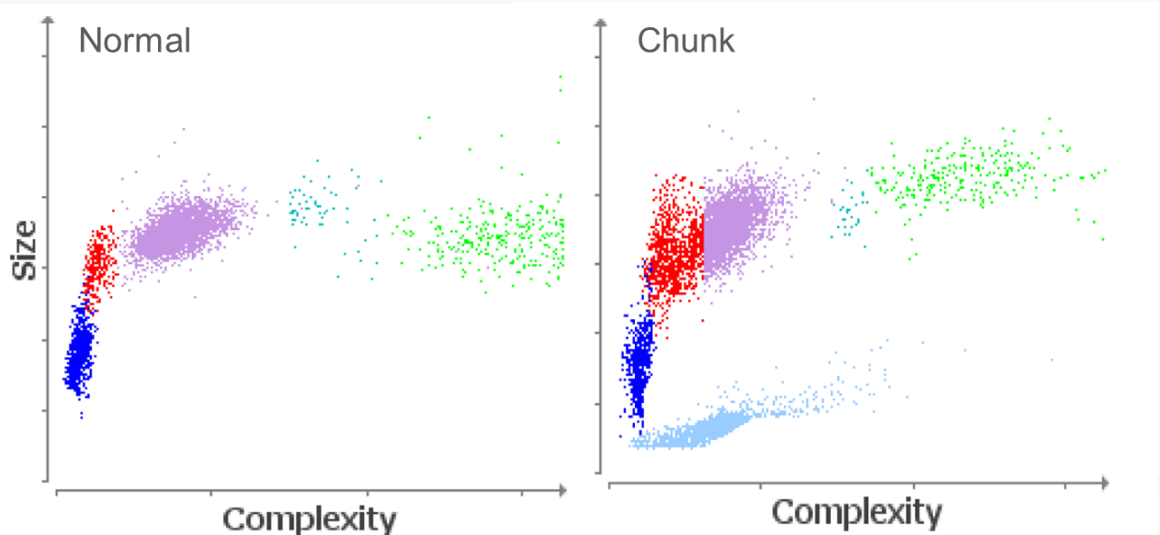


Perform a targeted assessment of blood morphology

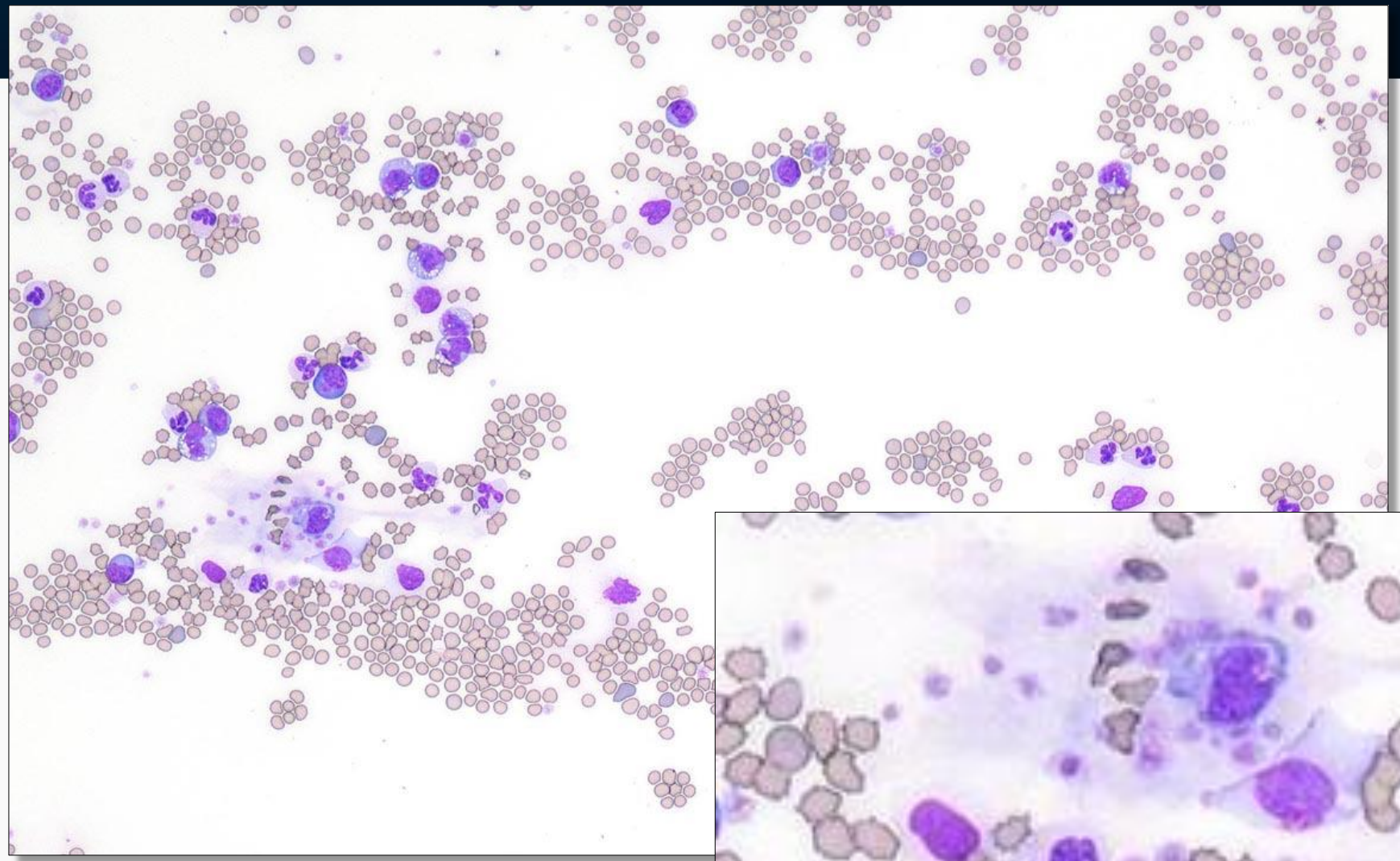


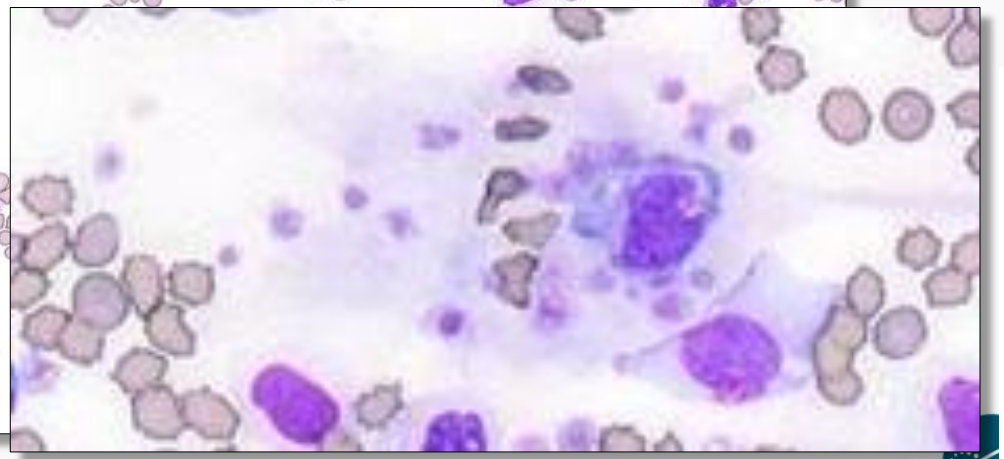
Moderate polychromasia
Moderate poikilocytosis

Intermediate to large mononuclear cells
Neutrophils



Perform a targeted assessment of blood morphology



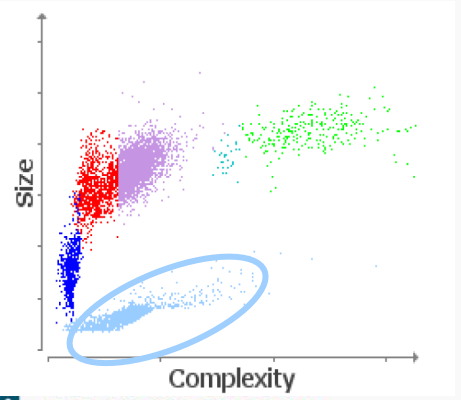


20x
Feathered edge

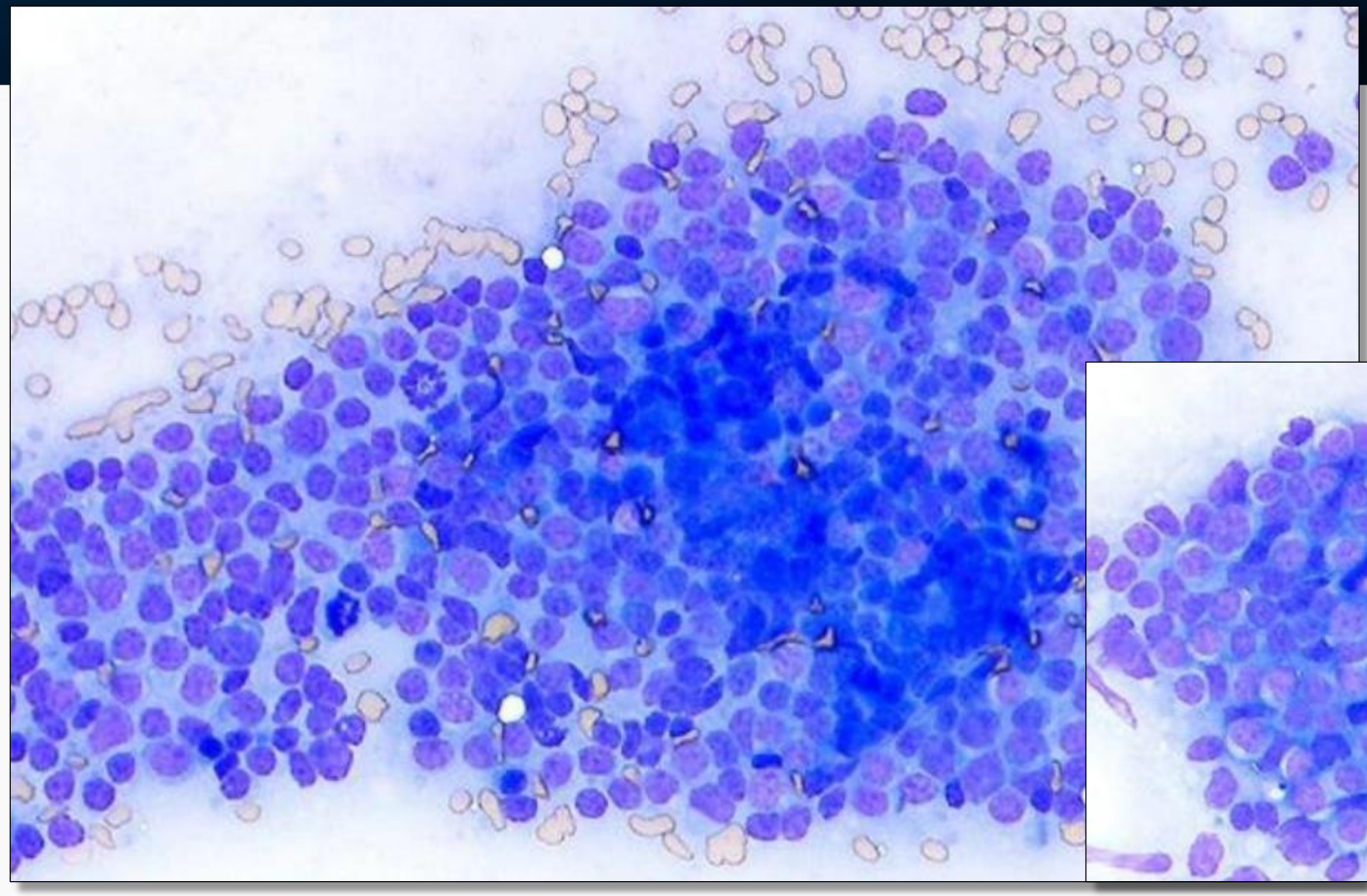
Small clumps of platelets

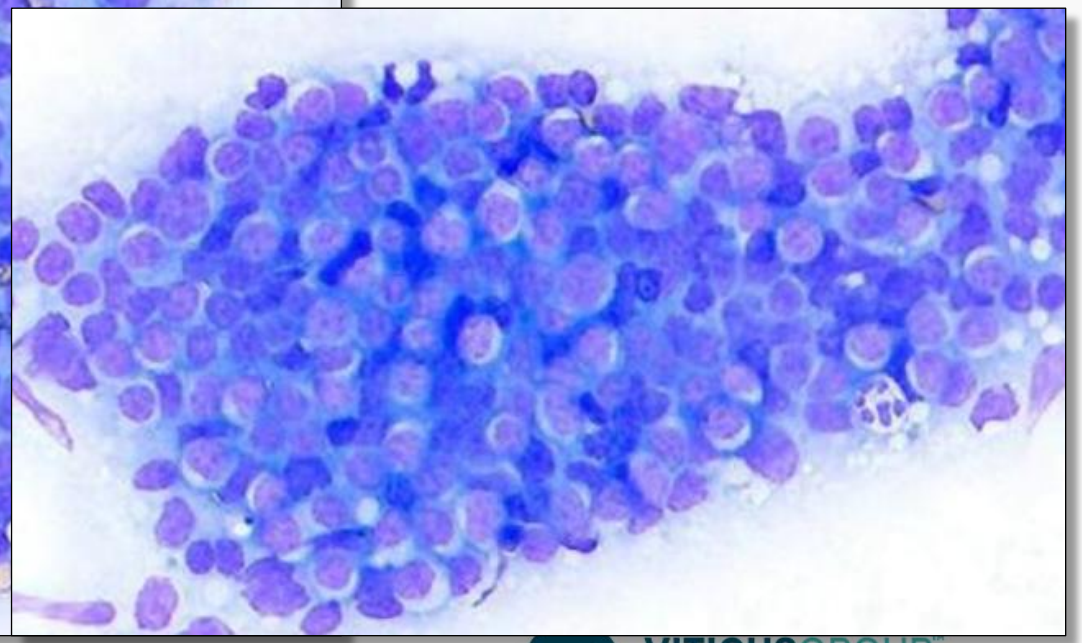
Moderate polychromasia

Many intermediate sized mononuclear cells



Chunk: 7-year-old, FS, Siamese cat





Enlarged mesenteric lymph node

Dx: Malignant lymphoma – intermediate cell size; stage V

Carl: 11-year-old, MN, Maltese dog

Clinical Presentation

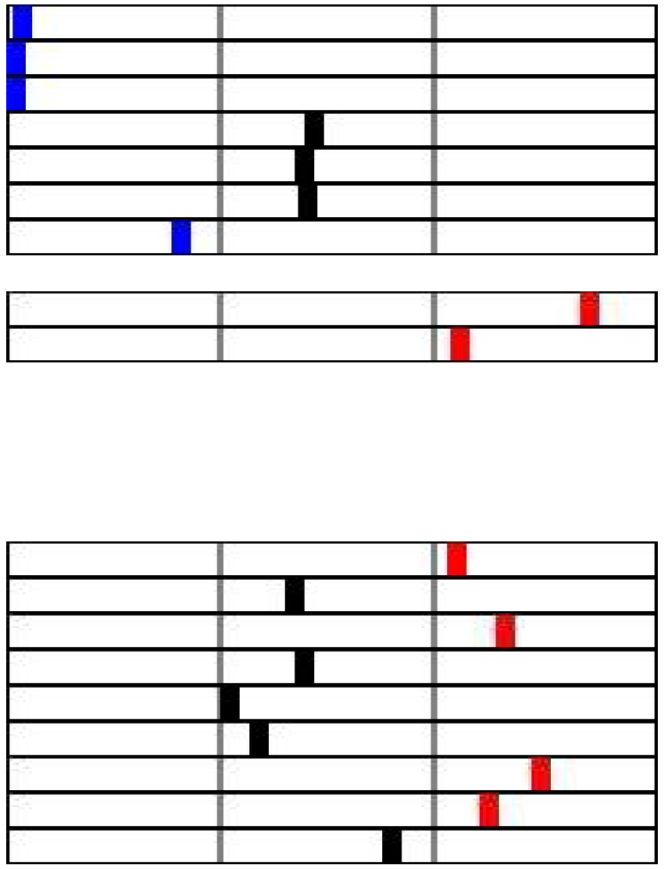
- Decreased appetite
- Lethargy

Diagnostic Summary

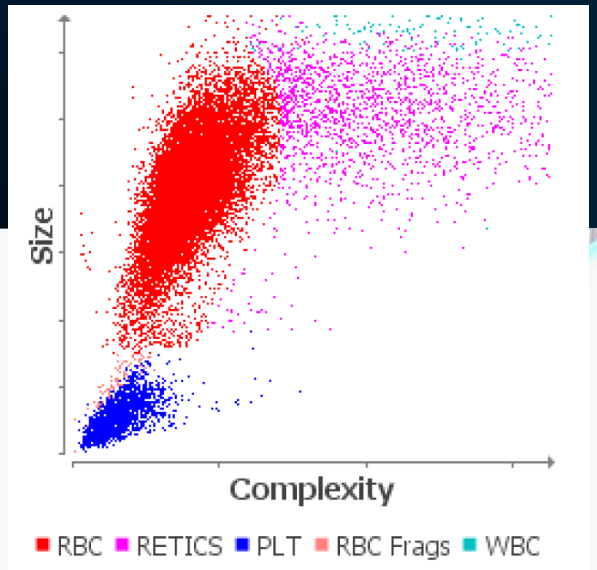
- Blood chemistry –
  - IDEXX SDMA® 17 µg/dl
  - Spec cPL® 1171 µg/L
  - ALP 1107 µ/L
  - Total bilirubin within normal limits
- Abdominal radiographs suggestive of enteritis
- CBC – refer to the following slides
- IDEXX SNAP®4Dx® test negative

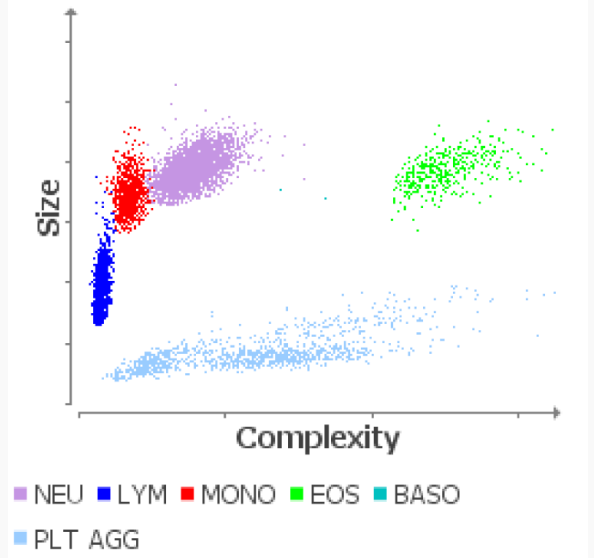
Carl: 11-year-old, MN, Maltese dog

Test	Results	Reference Interval	LOW / NORMAL / HIGH
ProCyte One (October 13, 2021 11:26 AM)			
RBC	2.30 M/µL	5.65 - 8.87	LOW
HCT	* 15.4 %	37.3 - 61.7	LOW
HGB	5.3 g/dL	13.1 - 20.5	LOW
MCV	* 67.0 fL	61.6 - 73.5	
MCH	23.1 pg	21.2 - 25.9	
MCHC	* 34.5 g/dL	32.0 - 37.9	
RDW	12.3 %	13.6 - 21.7	LOW
%RETIC	13.7 %		
RETIC	315.4 K/µL	10.0 - 110.0	HIGH
WBC	19.68 K/µL	5.05 - 16.76	HIGH
%NEU	74.1 %		
%LYM	12.6 %		
%MONO	10.6 %		
%EOS	2.7 %		
%BASO	0.0 %		
NEU	14.58 K/µL	2.95 - 11.64	HIGH
LYM	2.48 K/µL	1.05 - 5.10	
MONO	2.09 K/µL	0.16 - 1.12	HIGH
EOS	0.53 K/µL	0.06 - 1.23	
BASO	0.00 K/µL	0.00 - 0.10	
PLT	212 K/µL	148 - 484	
MPV	19.0 fL	8.7 - 13.2	HIGH
PDW	22.2 fL	9.1 - 19.4	HIGH
PCT	0.40 %	0.14 - 0.46	



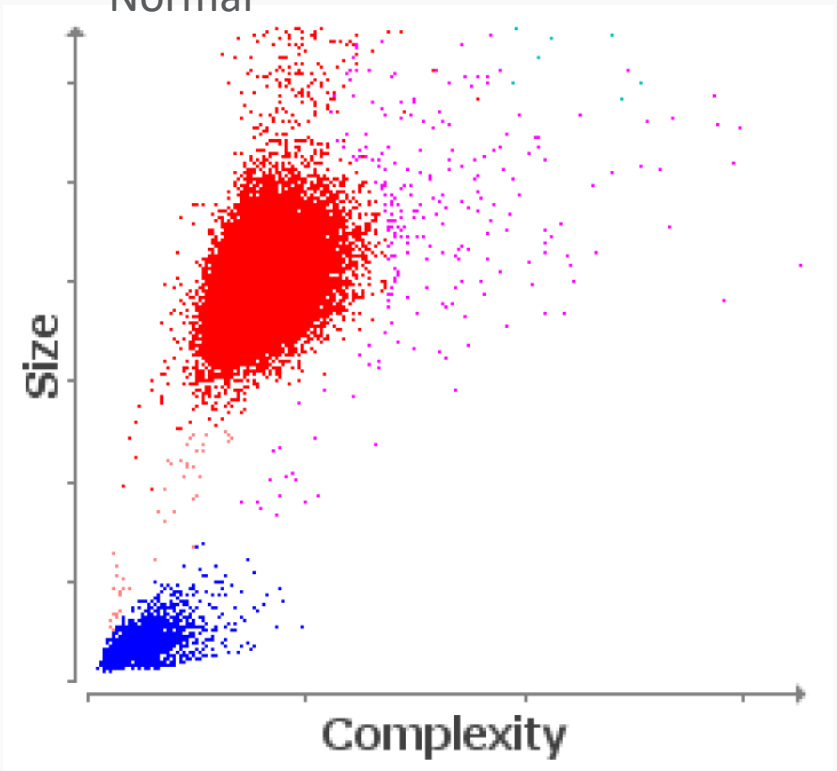
* Confirm with dot plot and/or blood film review.





Assessment of the Abnormal RBC/PLT Dot Plot

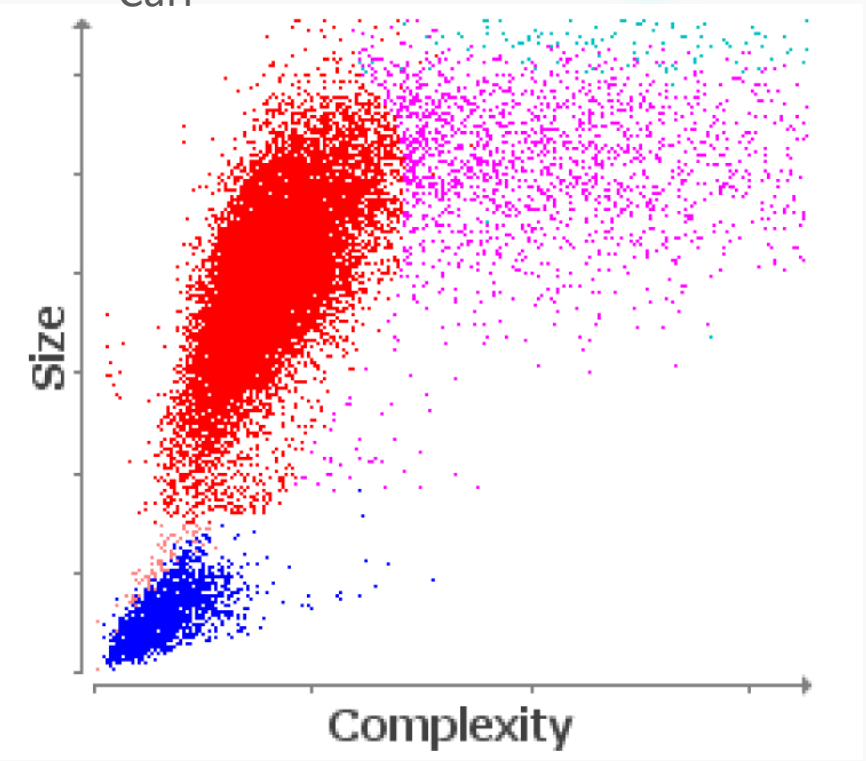
Normal



Is this a normal or abnormal dot plot?

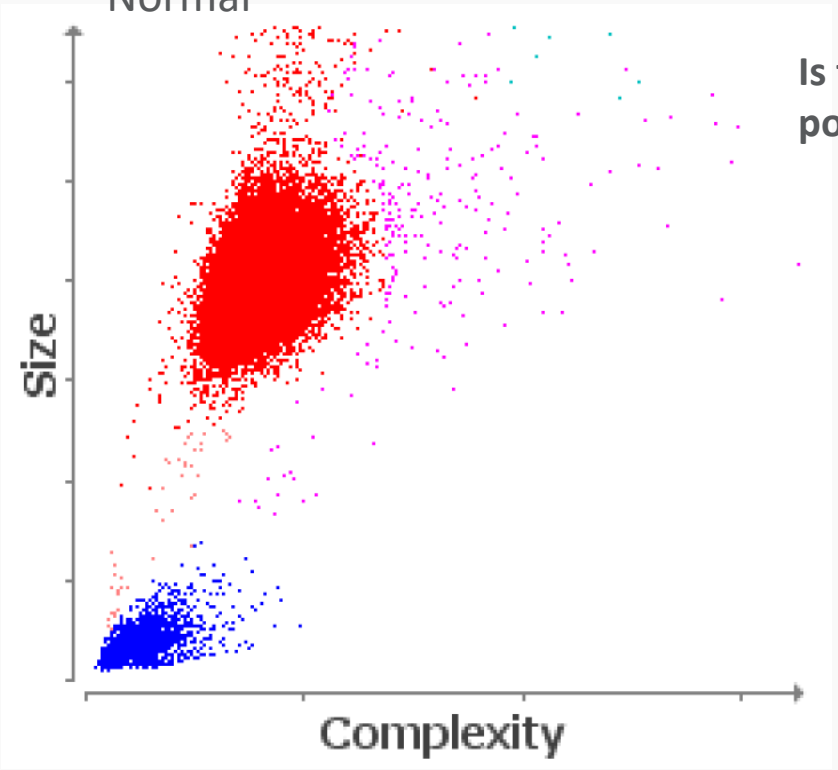
What is abnormal about it?

Carl



Assessment of the Abnormal RBC/PLT Dot Plot

Normal



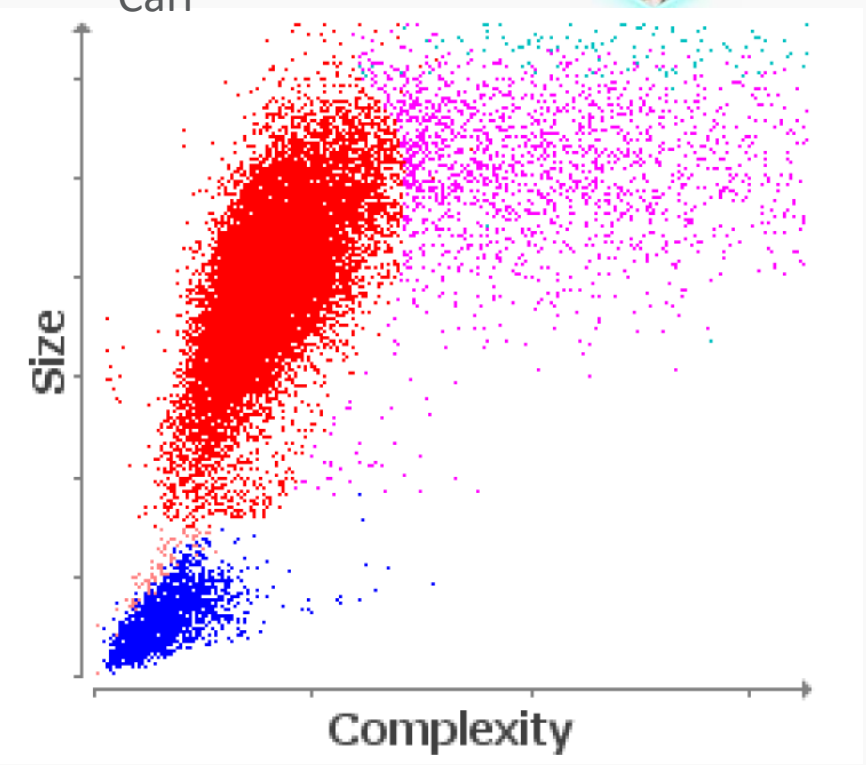
Is there good separation of the cell populations?

If YES, confidence in the automated numbers on the CBC

If NO, likely that biological morphologic changes are present

- Interpret numeric data with caution

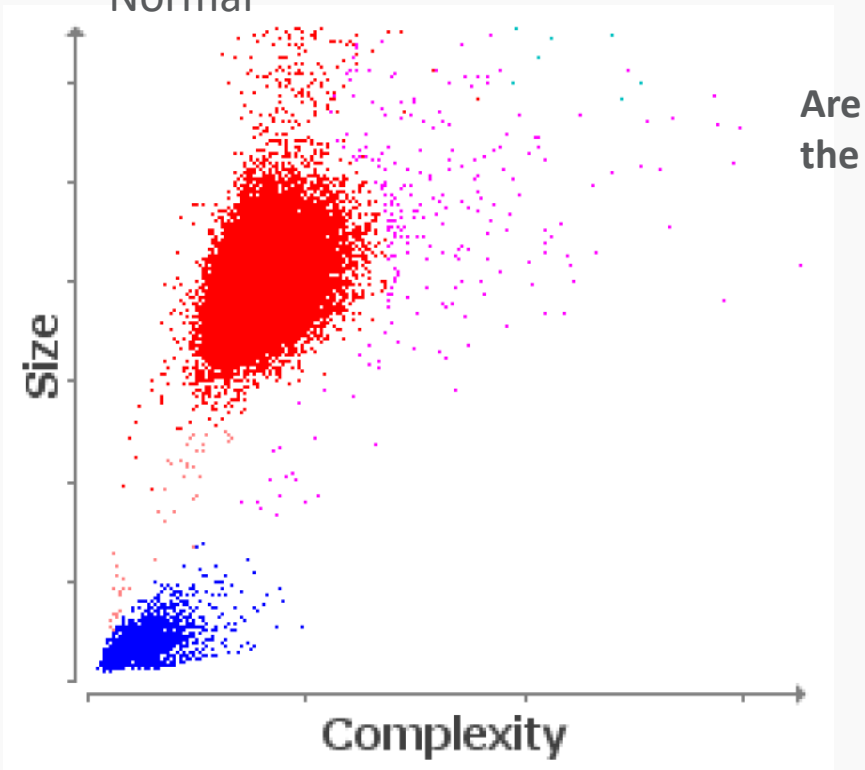
Carl



Here there is good separation of the cell populations.

Assessment of the Abnormal RBC/PLT Dot Plot

Normal

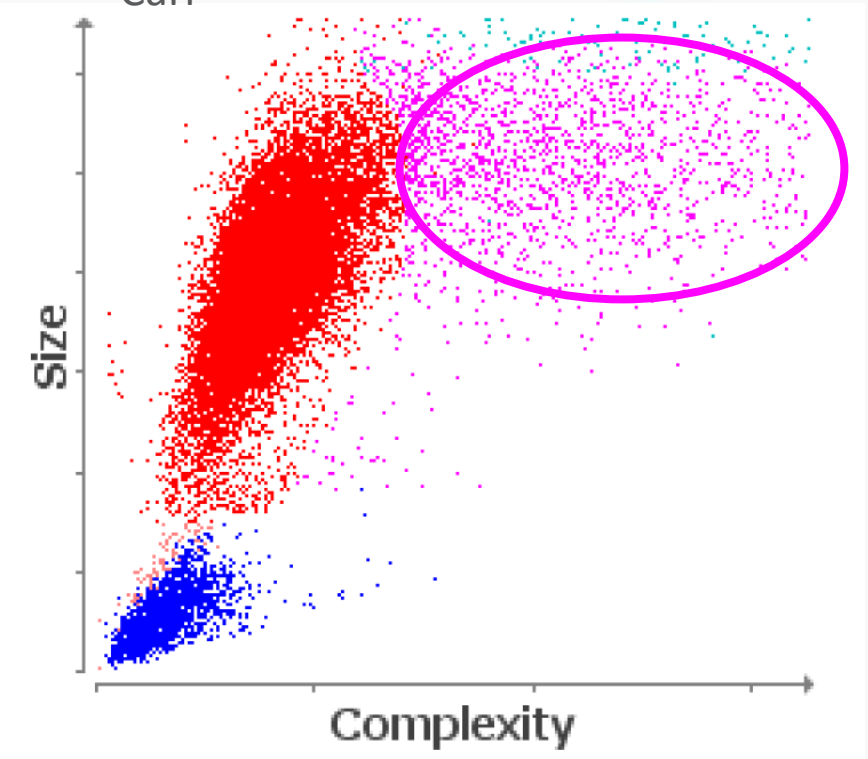


Are there changes in the densities of the cell clusters?

One can visually determine changes in quantity of

- reticulocytes
- platelets

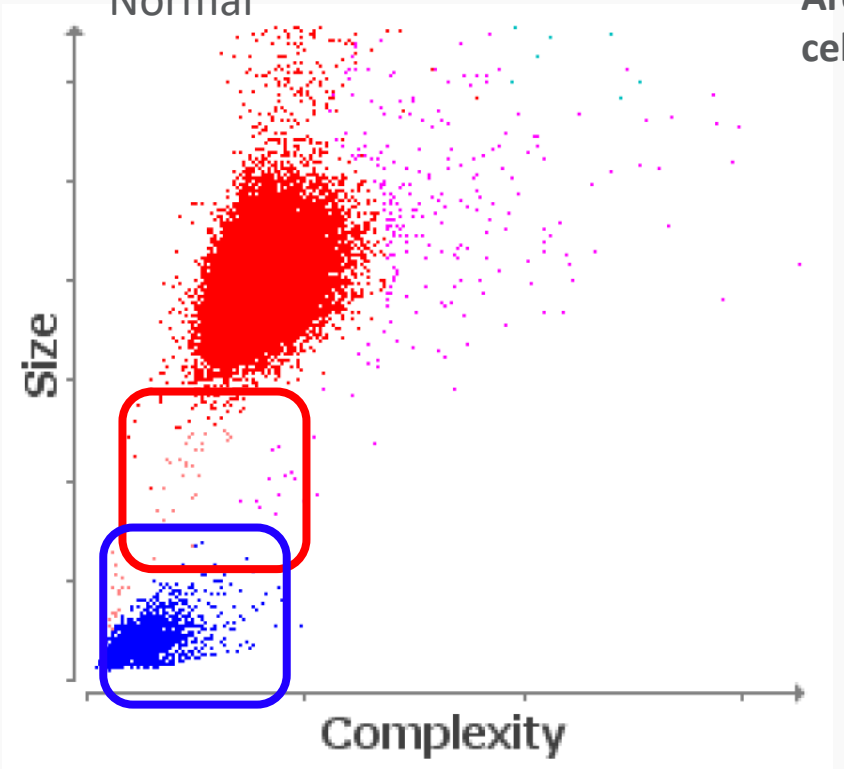
Carl



Here there is indication of reticulocytosis (increased density of dots in the pink circle).

Assessment of the Abnormal RBC/PLT Dot Plot

Normal

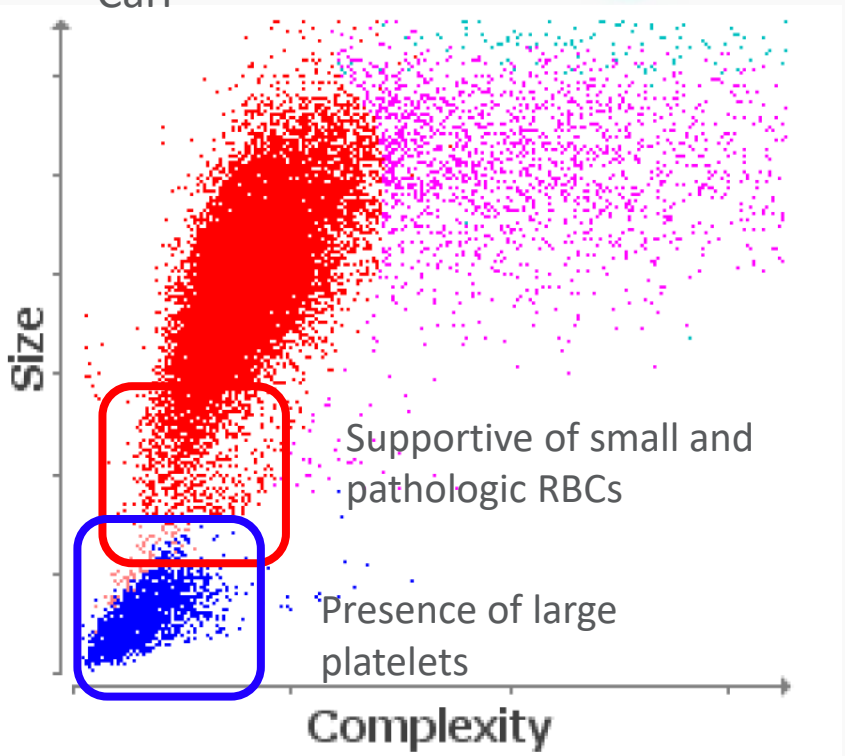


Are there changes in the location cells?

Biologic changes in cell morphology result in change in **the shape and location** of the cell clusters compared to normal

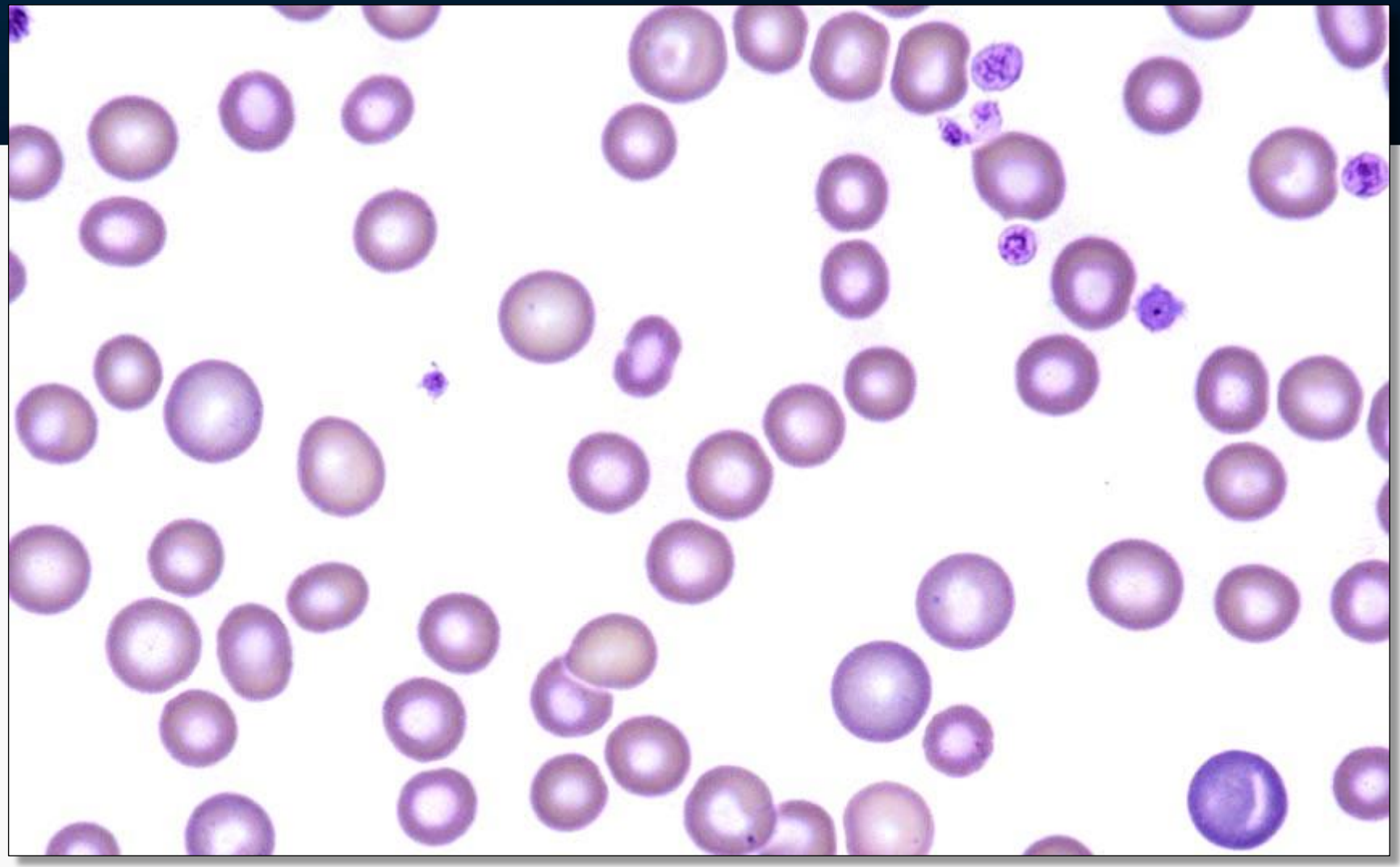
Directs a targeted assessment of blood morphology

Carl



Abnormal positioning of digitized events supports abnormal cell morphology – ***blood morphology must be evaluated***

Perform a targeted assessment of blood morphology



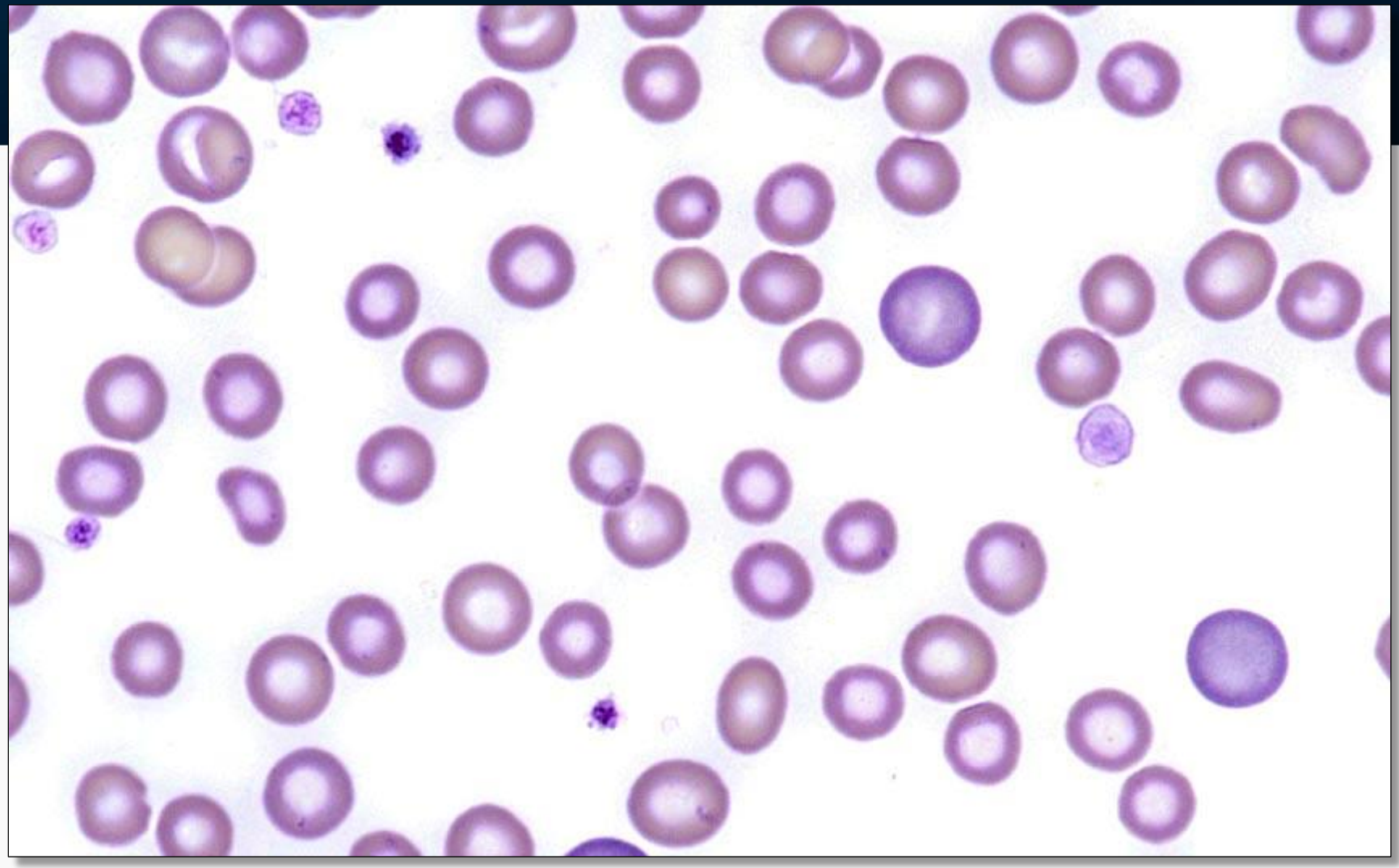
100x

Monolayer of blood film

Large platelets

Moderate anisocytosis
Moderate polychromasia
Increased central pallor

Perform a targeted assessment of blood morphology



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Increased central pallor

Carl: 11- year-old, MN, Maltese dog

Test	Results	Reference Interval	LOW / NORMAL / HIGH
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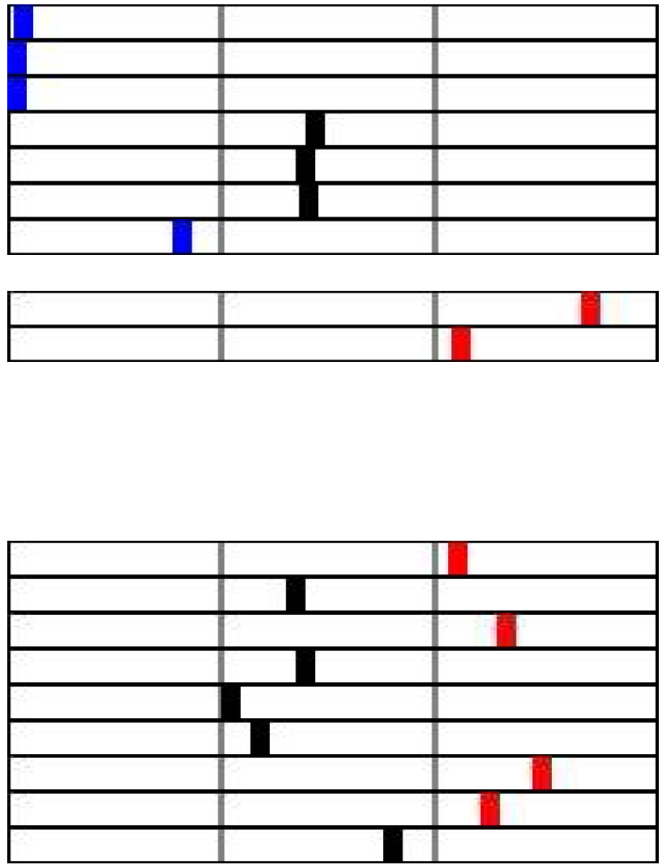
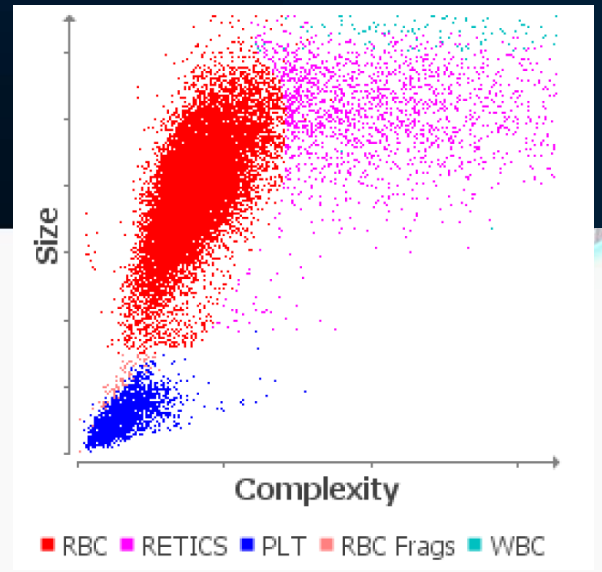
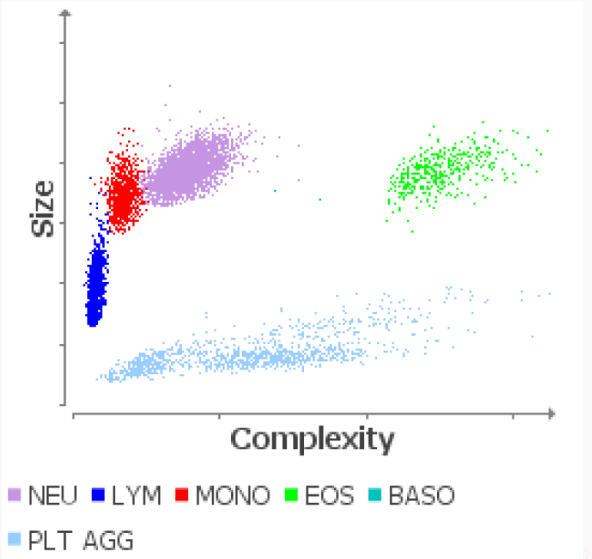
%LYM	12.6 %		
%MONO	10.6 %		
%EOS	2.7 %		
%BASO	0.0 %		
NEU	14.58 K/µL	2.95 - 11.64	HIGH
LYM	2.48 K/µL	1.05 - 5.10	
MONO	2.09 K/µL	0.16 - 1.12	HIGH
EOS	0.53 K/µL	0.06 - 1.23	
BASO	0.00 K/µL	0.00 - 0.10	
PLT	212 K/µL	148 - 484	
MPV	19.0 fL	8.7 - 13.2	HIGH
PDW	22.2 fL	9.1 - 19.4	HIGH
PCT	0.40 %	0.14 - 0.46	

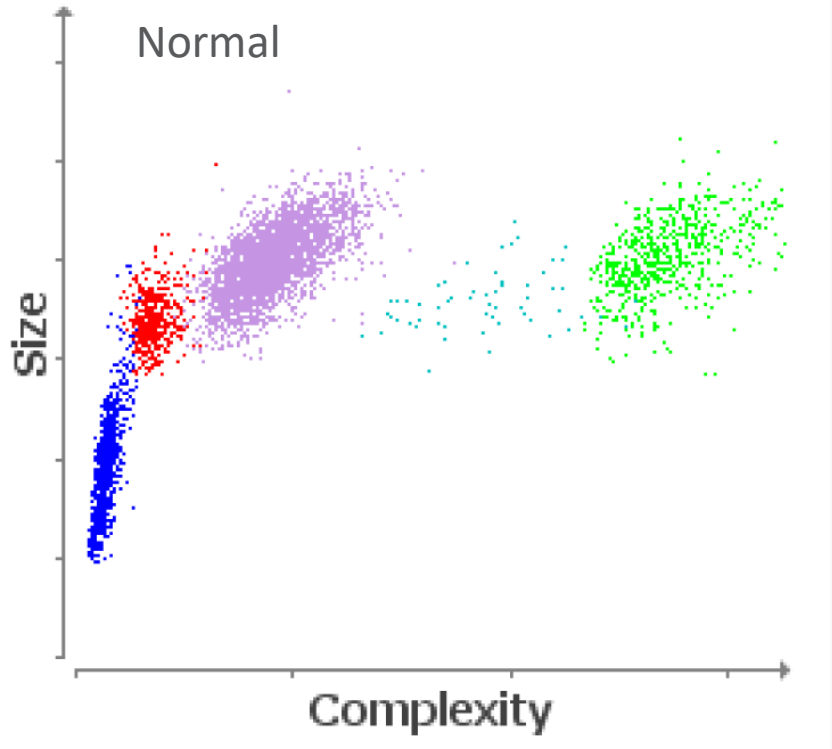


* Confirm with dot plot and/or blood film review.



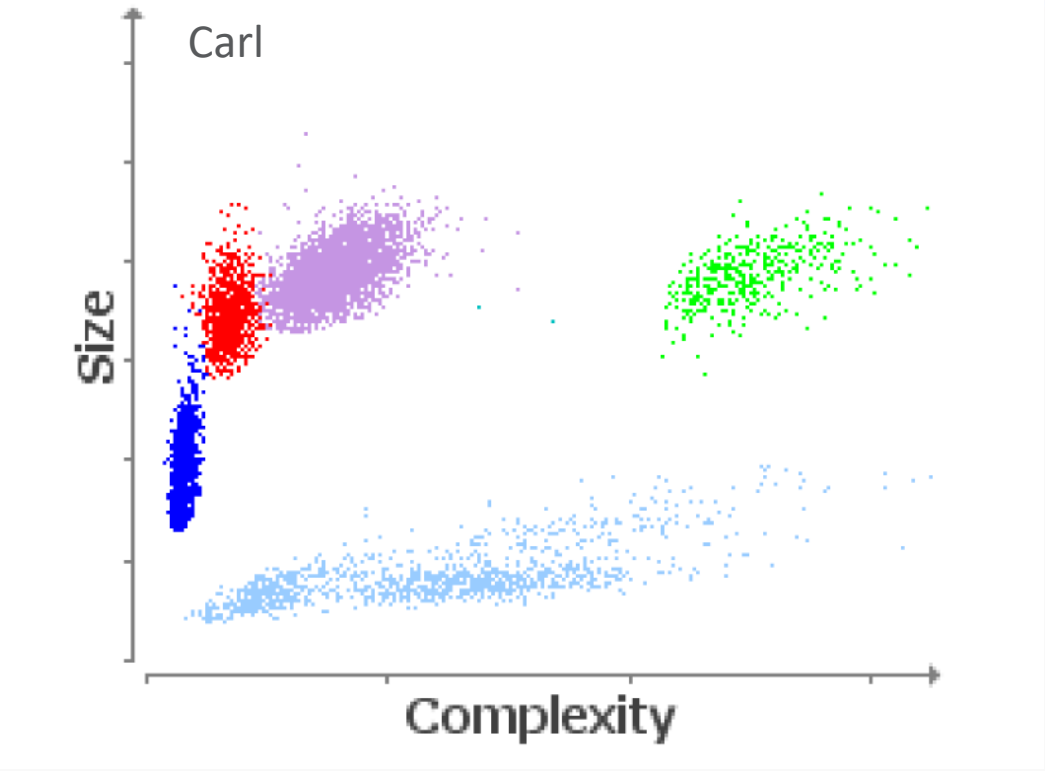


Assessment of the Abnormal WBC Dot Plot

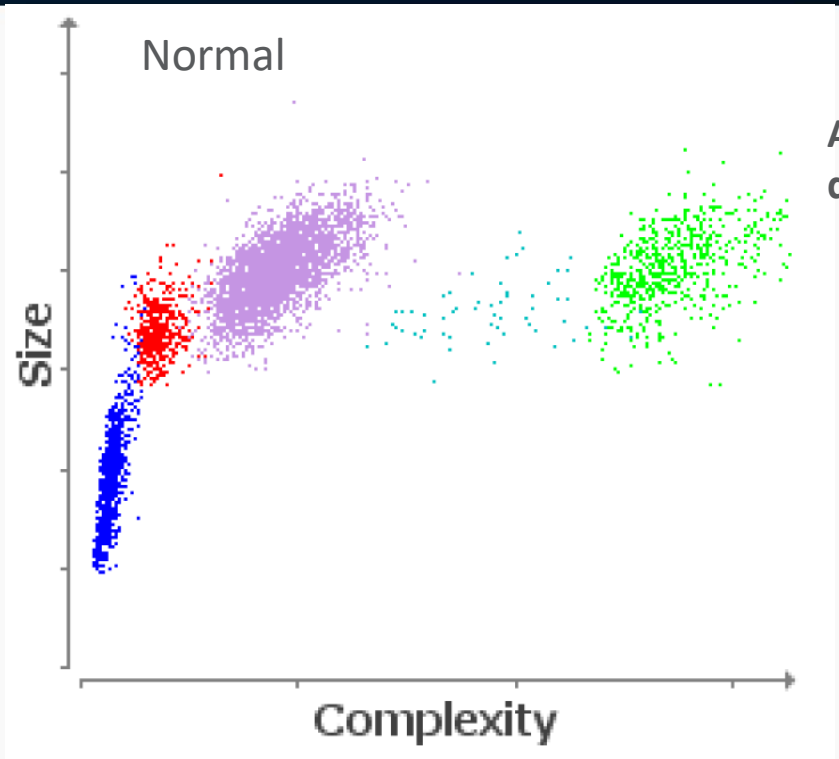


Is this a normal or abnormal dot plot?

What is abnormal about it?



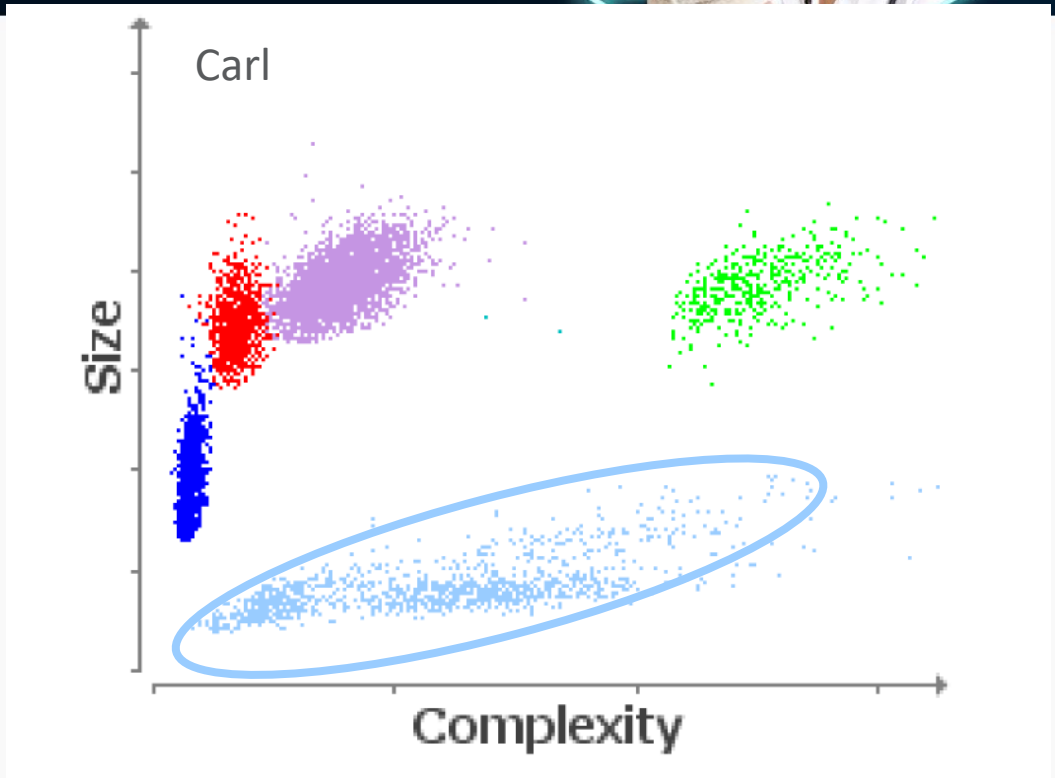
Assessment of the Abnormal WBC Dot Plot



Are there changes in the densities of the cell clusters?

One can visually determine changes in quantity of

- The different WBCs
- Presence of platelet clumps



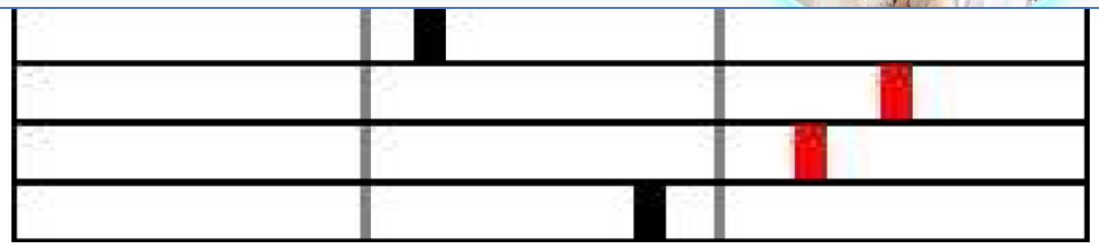
Carl: 11-year-old, MN, Maltese dog

Test	Results	Reference Interval		LOW	NORMAL	HIGH
WBC	19.68 K/µL	5.05 - 16.76	HIGH			
NEU	14.58 K/µL	2.95 - 11.64	HIGH			
LYM	2.48 K/µL	1.05 - 5.10				
MONO	2.09 K/µL	0.16 - 1.12	HIGH			
EOS	0.53 K/µL	0.06 - 1.23				
BASO	0.00 K/µL	0.00 - 0.10				

- Primary changes
  - Mild leukocytosis
  - Neutrophilia and monocytosis – most likely chronic inflammation

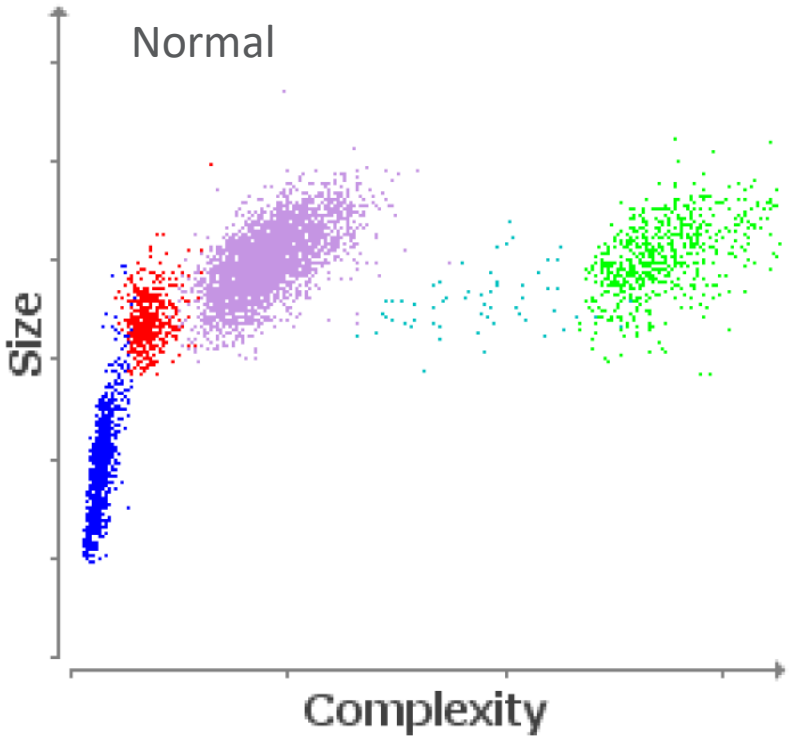
Carl: 11-year-old, MN, Maltese dog

PLT	212 K/µL	148 - 484	
MPV	19.0 fL	8.7 - 13.2	HIGH
PDW	22.2 fL	9.1 - 19.4	HIGH
PCT	0.40 %	0.14 - 0.46	



- Primary changes
  - Increased platelet indices of MPV and PDW
  - These values can be altered by the presence of platelet aggregates (doublets or clumping)

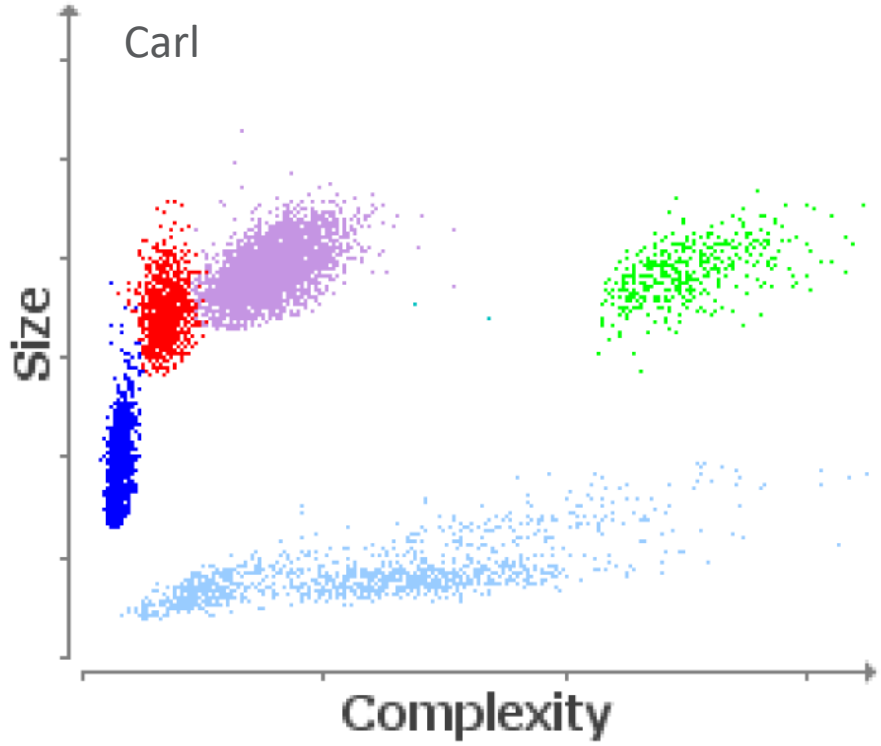
Assessment of the Abnormal WBC Dot Plot



Are there changes in the location of the cells?

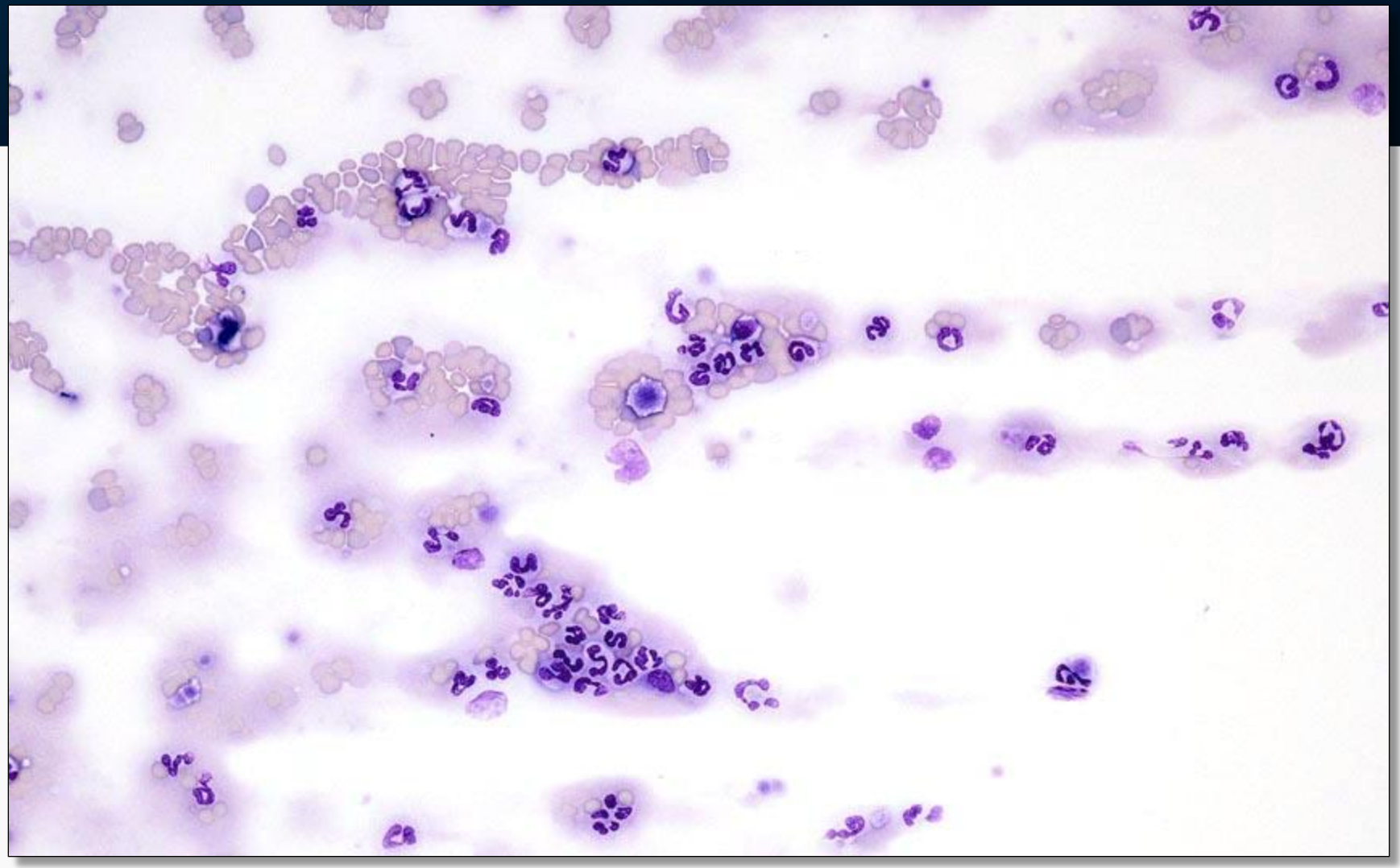
Biologic changes in cell morphology result in change in **the shape and location** of the cell clusters compared to normal

Directs a targeted assessment of blood morphology



Usual positioning of digitized events supports normal cell morphology

Perform a targeted assessment of blood morphology



20x

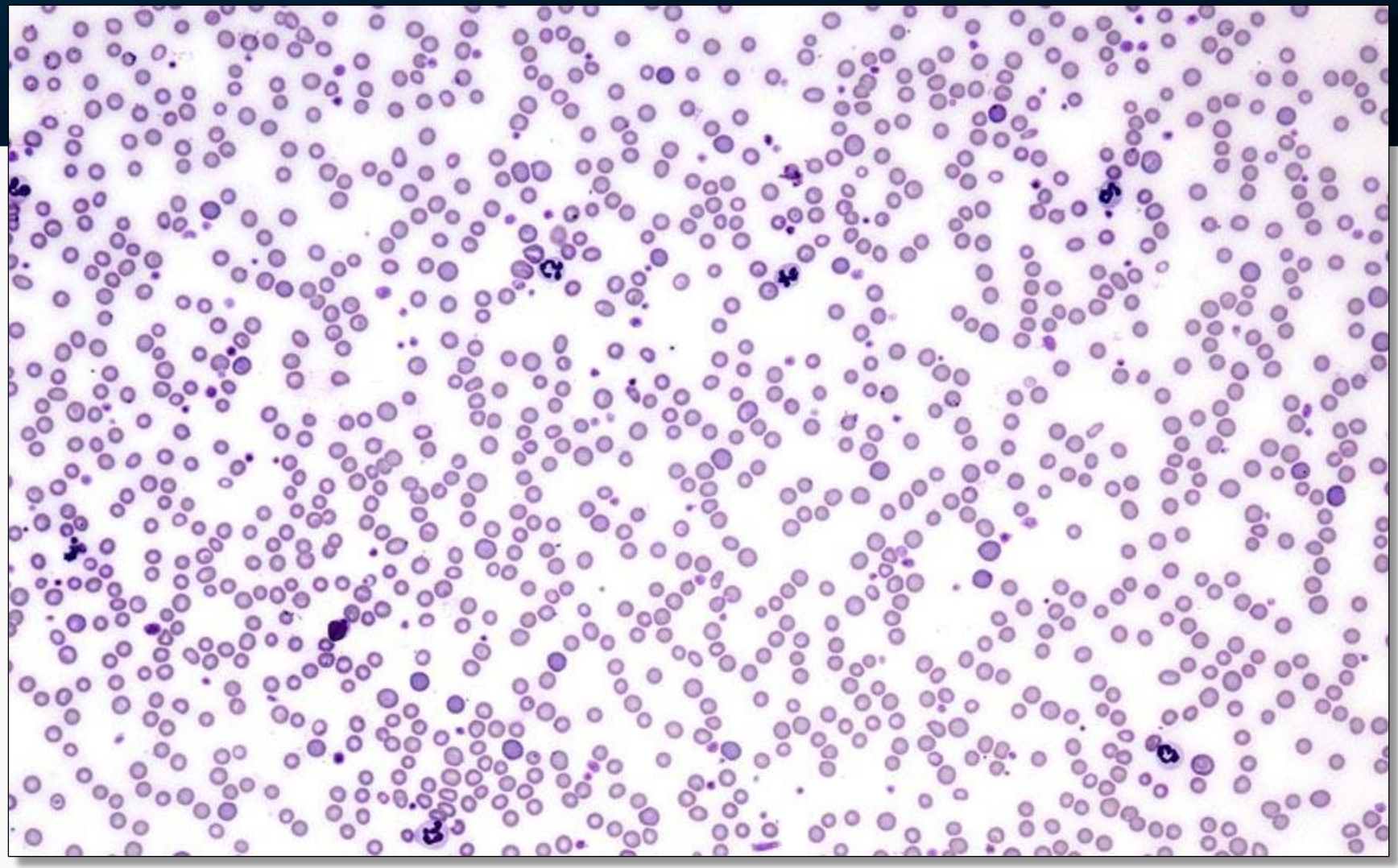
Feathered edge of blood film

Possible decreased platelets
Large and giant platelets

Moderate polychromasia

Normal to slightly increased leukocyte density

Perform a targeted assessment of blood morphology



20x

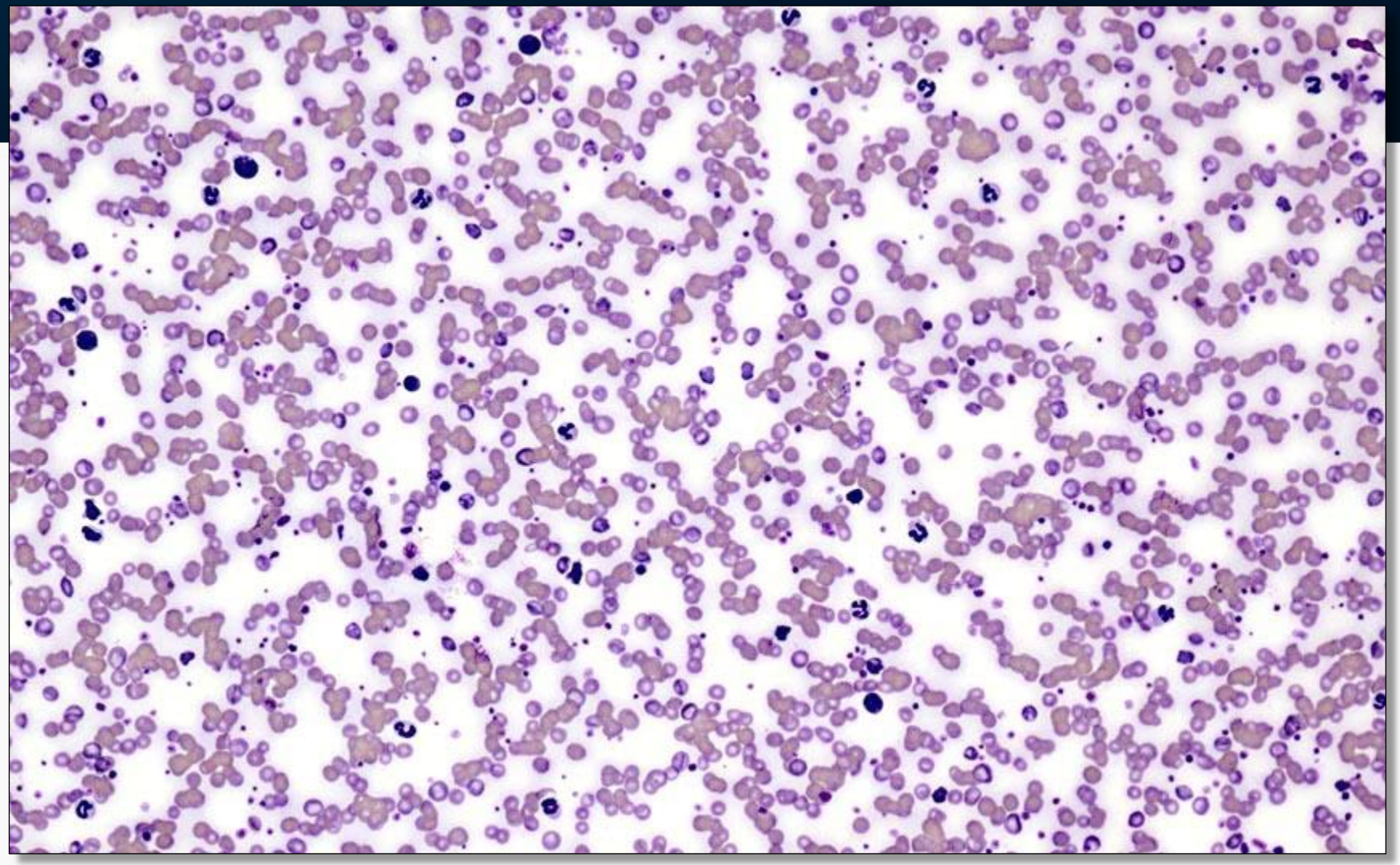
Monolayer of blood film

Large platelets

Moderate anisocytosis
Moderate polychromasia
Possible increased central pallor

Normal leukocyte density
Mostly mature neutrophils

Perform a targeted assessment of blood morphology



20x

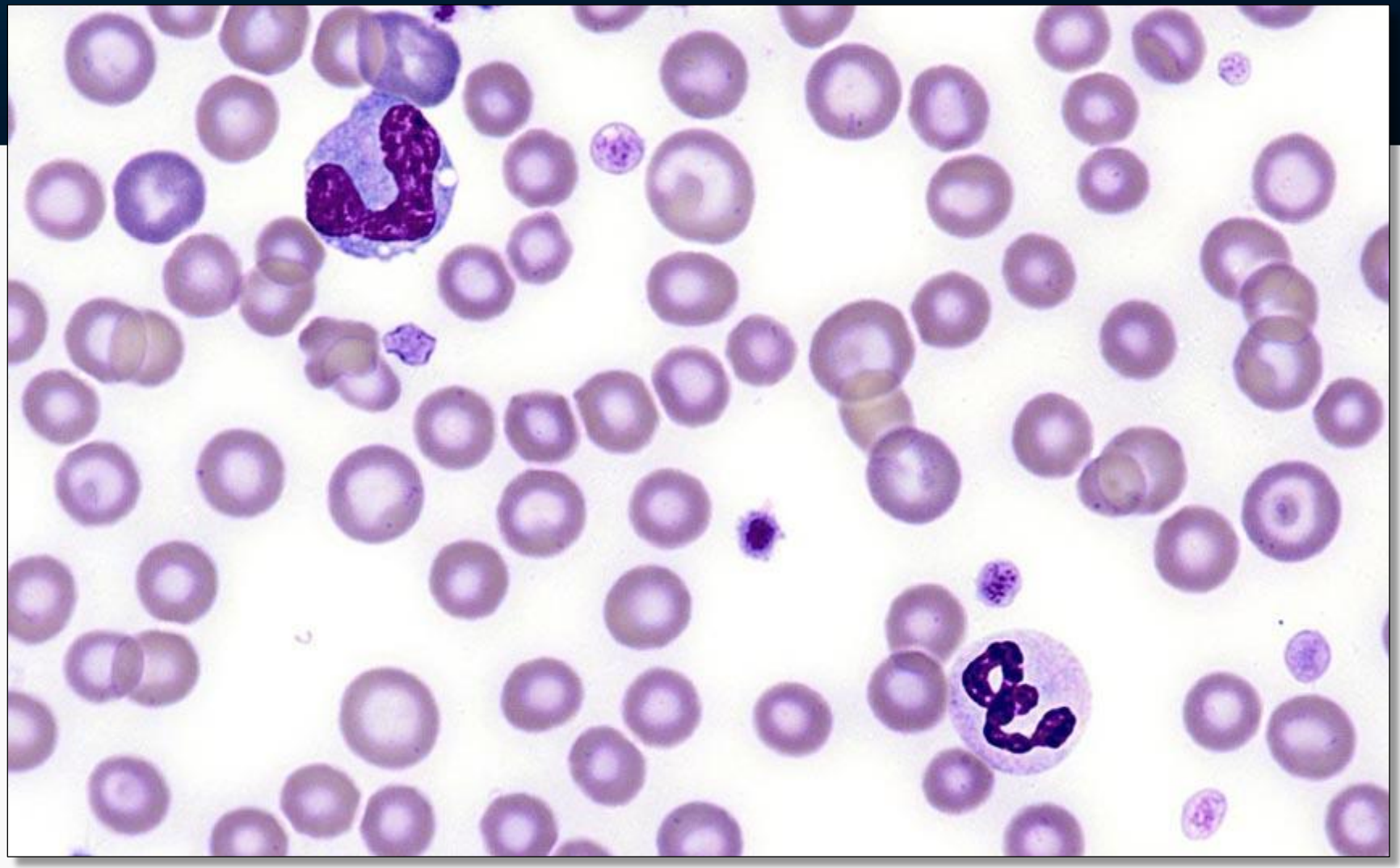
Body of blood film

Large platelets

Normal to slightly increased Rouleaux
Moderate anisocytosis
Moderate polychromasia
Possible increased central pallor

Normal leukocyte density
Mostly mature neutrophils

Perform a targeted assessment of blood morphology



100x

Monolayer of blood film

Low within reference interval limit platelet numbers
Large platelets

Moderate anisocytosis
Moderate polychromasia
Possible increased central pallor

Mature neutrophil
Monocyte

Carl: 11-year-old, MN, Maltese dog

- Strongly regenerative anemia
- Mild inflammatory leukogram
- Small red blood cells indicated by dot plots
- Review of blood morphology reveals
  - Decreased central pallor of red blood cells
  - Confirms regenerative anemia
  - Mature neutrophils
  - No spherocytosis

Iron deficiency and GI blood loss

Red Blood Cell and Platelet Dot Plots

- **Changes in the density of the cell clusters**
  - Reticulocytosis
  - Absence of reticulocytosis (lack of a regenerative response when anemia present)
  - Thrombocytosis
  - Thrombocytopenia
- **Changes in the shape and position of the cells**
  - Abnormal cell morphology
  - Large or doublet platelets

Photo credit: Kim Yore, Westbrook, Maine

White Blood Cell Dot Plots

- **Changes in the density of the cell clusters**
  - Leukopenia
  - Leukocytosis
- **Changes in the shape and position of the cells**
  - Abnormal cell morphology
- **Presence of platelet clumps**

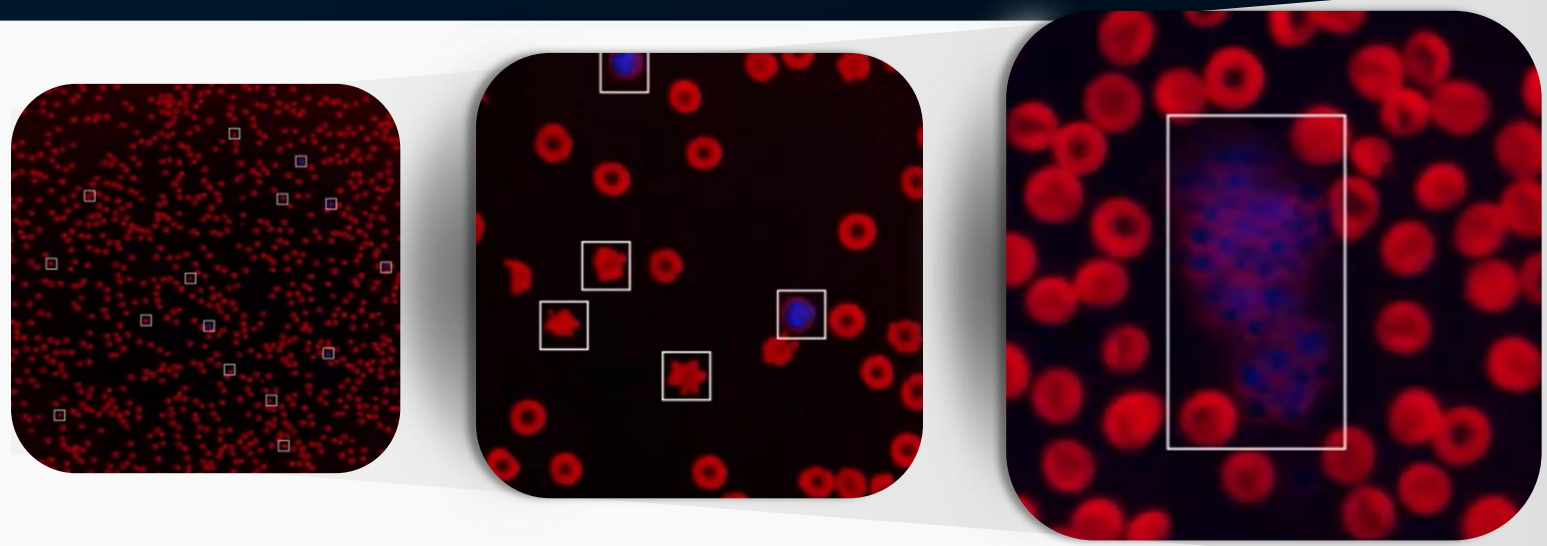
Photo credit: Kim Yore, Westbrook, Maine

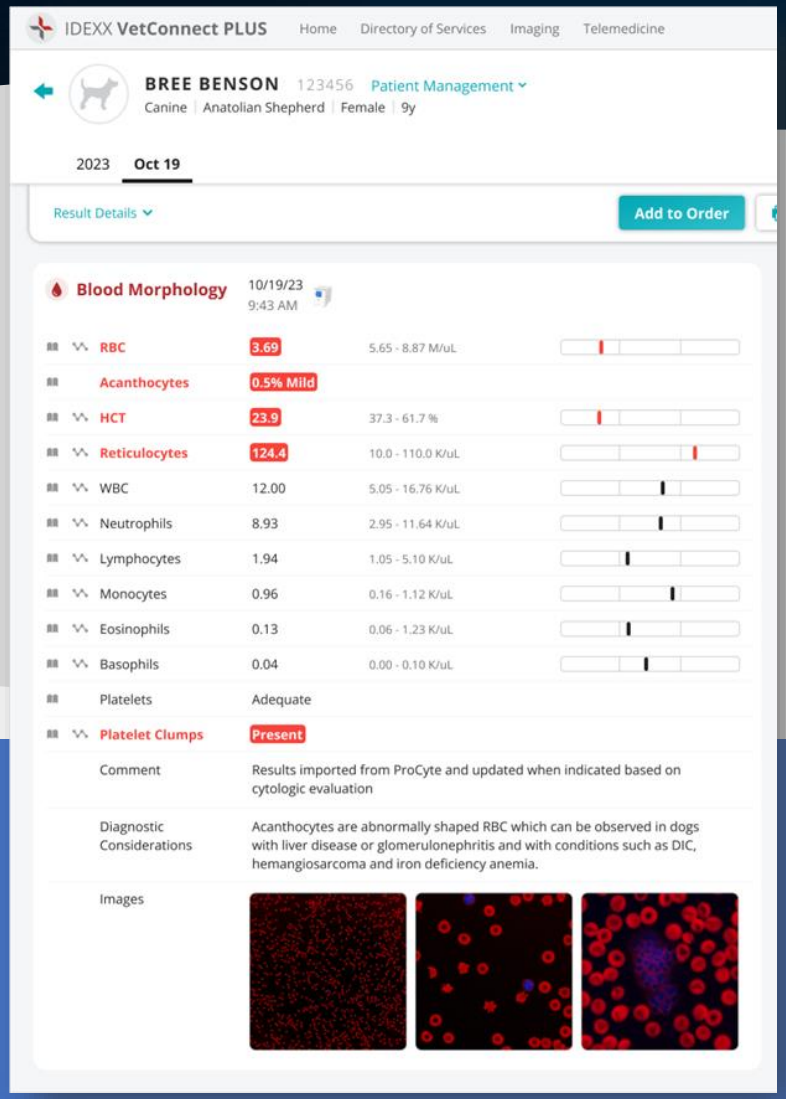
Targeted Evaluation of Blood Morphology

Dot plots tell you when you must evaluate blood morphology

- Even when numeric values of the CBC are normal, dot plots can direct assessment of morphological changes in the CBC
- Look for changes in the usual position and poor separation of cell clusters
- Good separation of cell clusters and usual positioning of digitized events supports normal cell morphology

IDEXX inVue Dx™ analyzer: automated quantification, classification, and interpretation of blood morphology





Deep-Learning Models Trained by IDEXX Reference Lab Pathologists

Summary

Hematology is more than the numbers on the report

- Essential parts of a complete blood count (CBC)
  - Numeric values
  - Graphical data
  - Interpretive aids
- Dot plots and blood morphology evaluation increase clinical insights
- Learn to recognize normal from abnormal
- Blood morphology indicated in all unwell patients and when abnormalities seen on CBC