

Scatterplot Interpretation

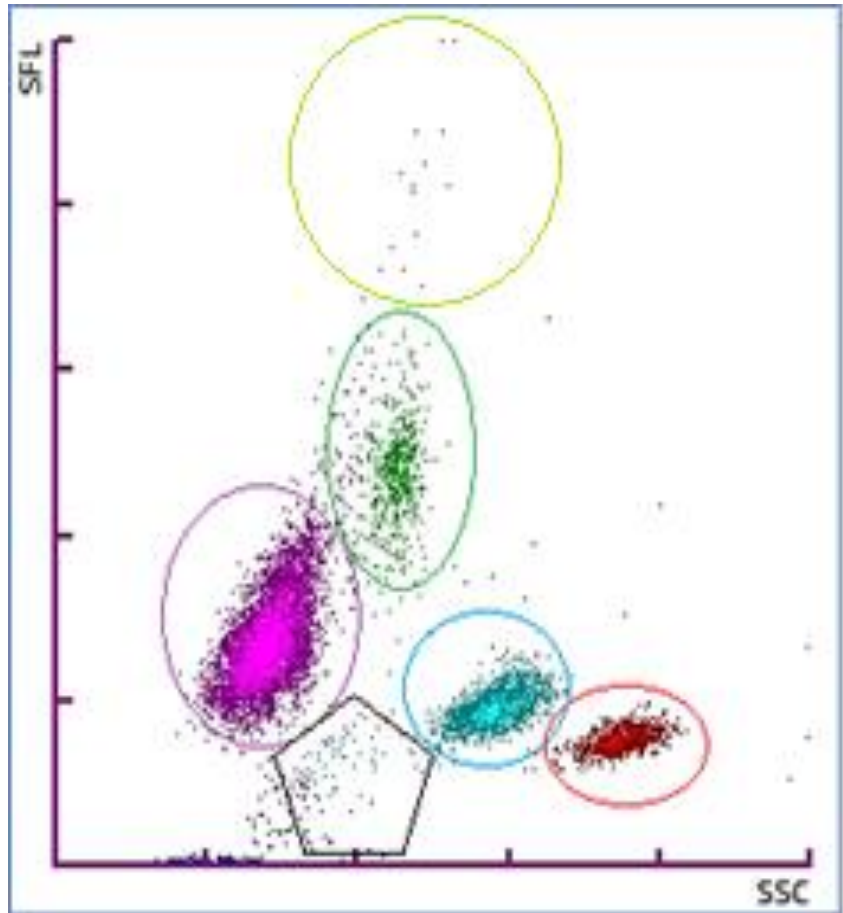
Sysmex XN series

October 2022

Background and context

- In a recent event the laboratory reported an incorrect and clinically significant differential with a marked neutropenia.
- The purpose of this education material is to empower the scientist with tools to enable accurate reporting of the differential
 - Interpretation of scatter plots
 - Suggestions for blood film scanning
 - Suggestions for differential counting

This is the scatterplot from the patient involved



Neutrophils

Lymphocytes

Monocytes

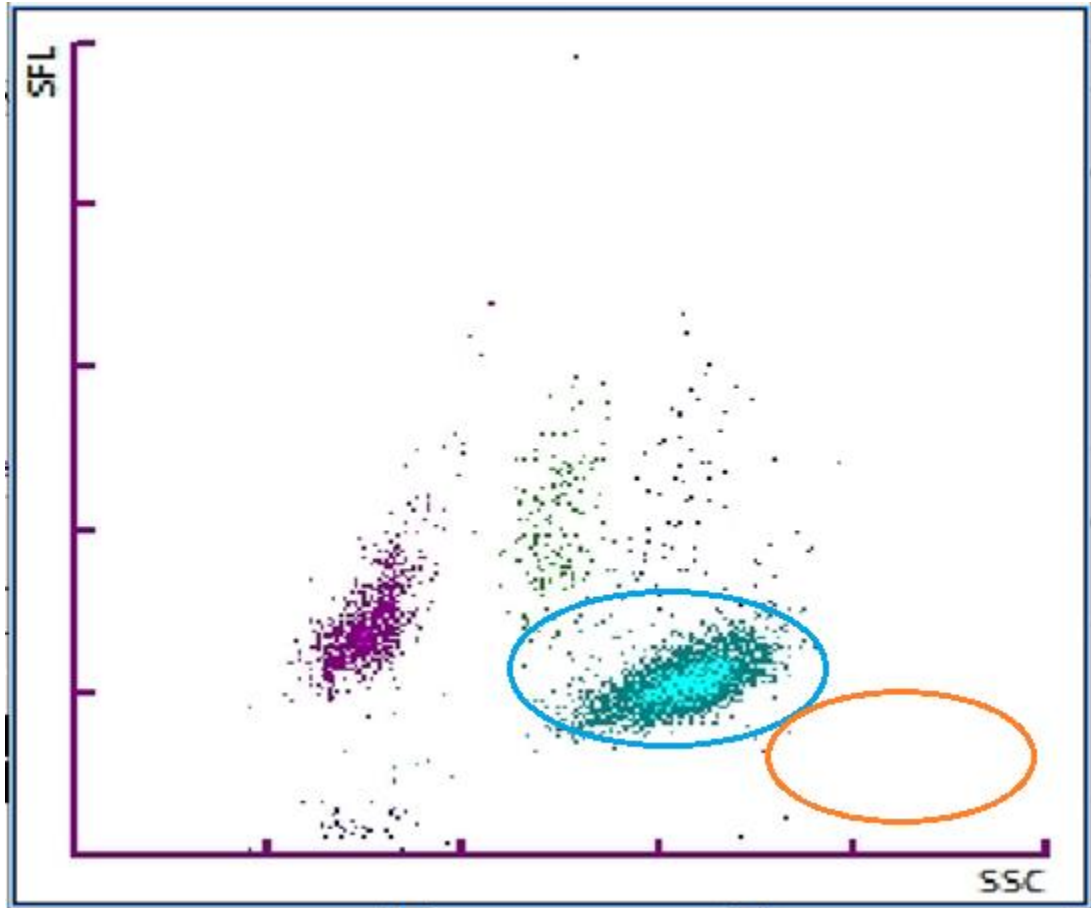
Eosinophils

Basophils

Reactive lymphocytes

Junk

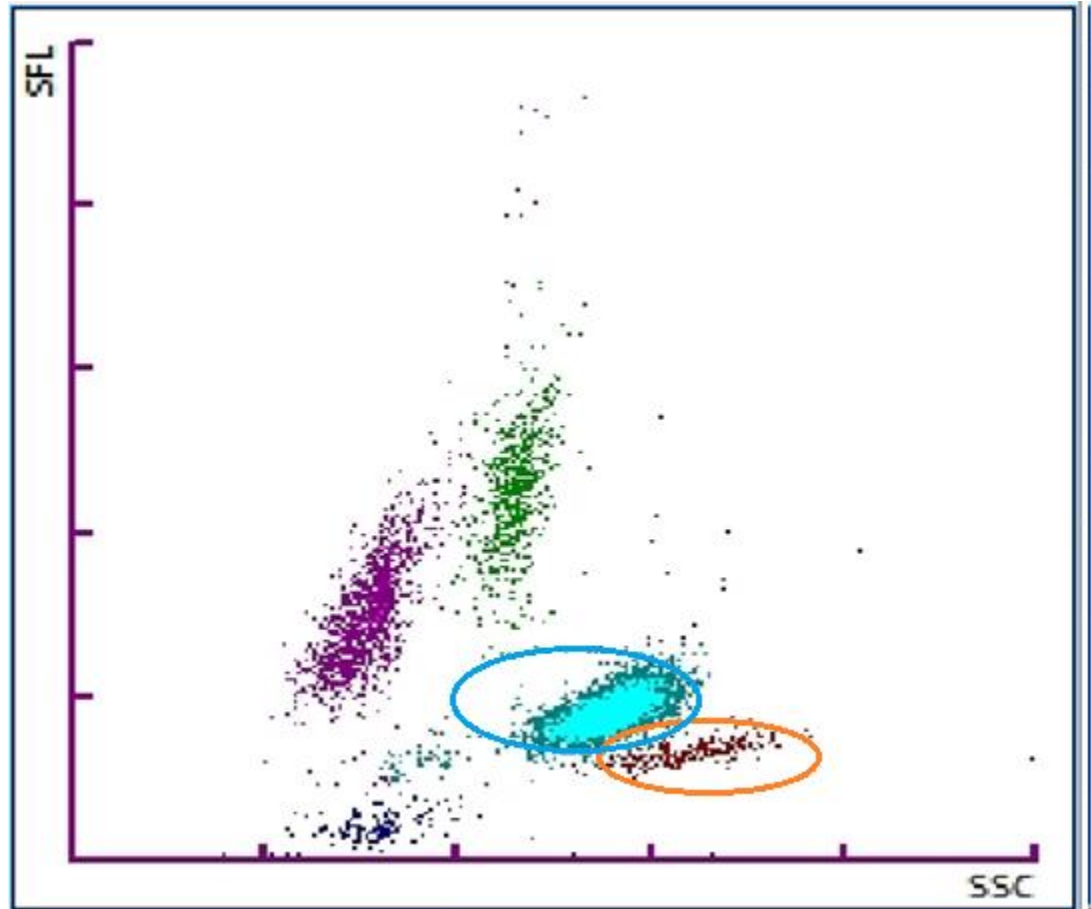
Example 1 relative Neutrophilia



- **Neutrophils 75%**
- **Lymphocytes 21%**
- **Monocytes 4%**
- **Eosinophils 0%**
- *Basophils 0%*

Note the intensity of the neutrophil and eosinophil clusters

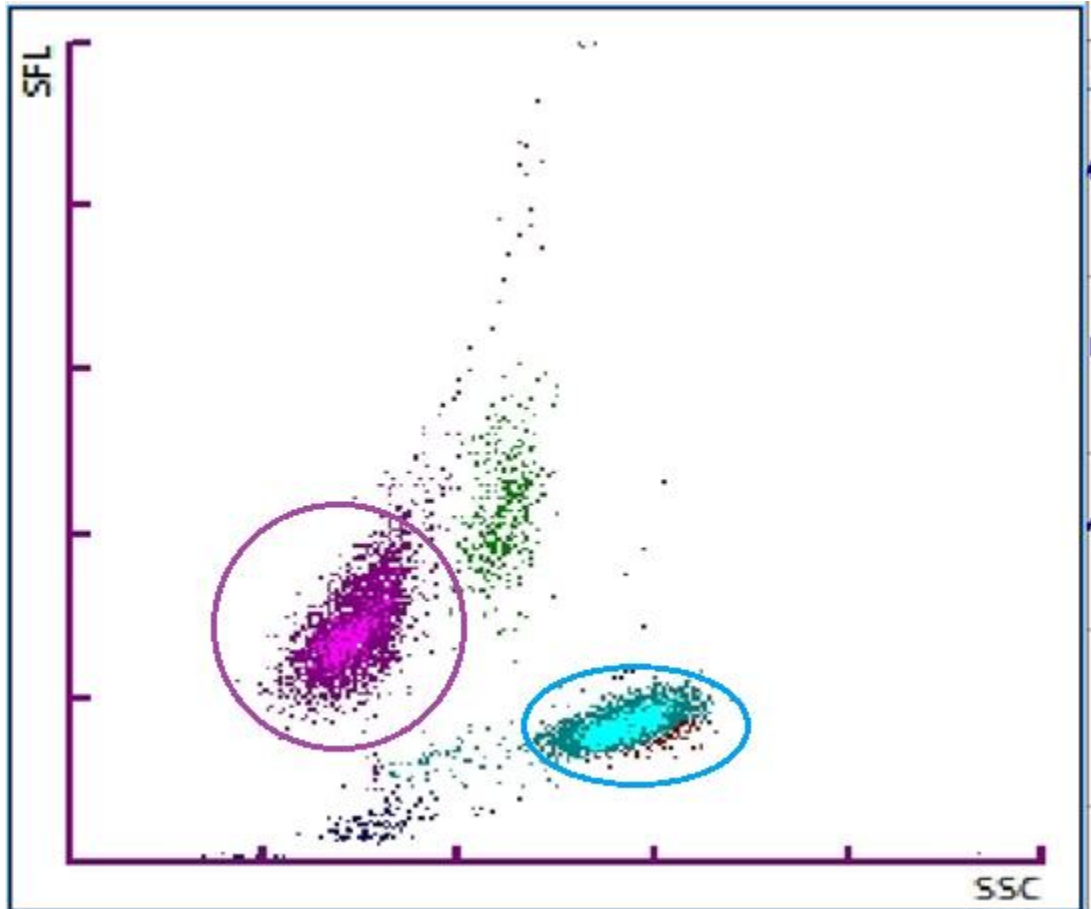
Example 2 relative Neutrophilia 3% Eosinophils



- **Neutrophils 70%**
- **Lymphocytes 18%**
- **Monocytes 9%**
- **Eosinophils 3%**
- *Basophils 1%*

Note the intensity of the neutrophil and eosinophil clusters

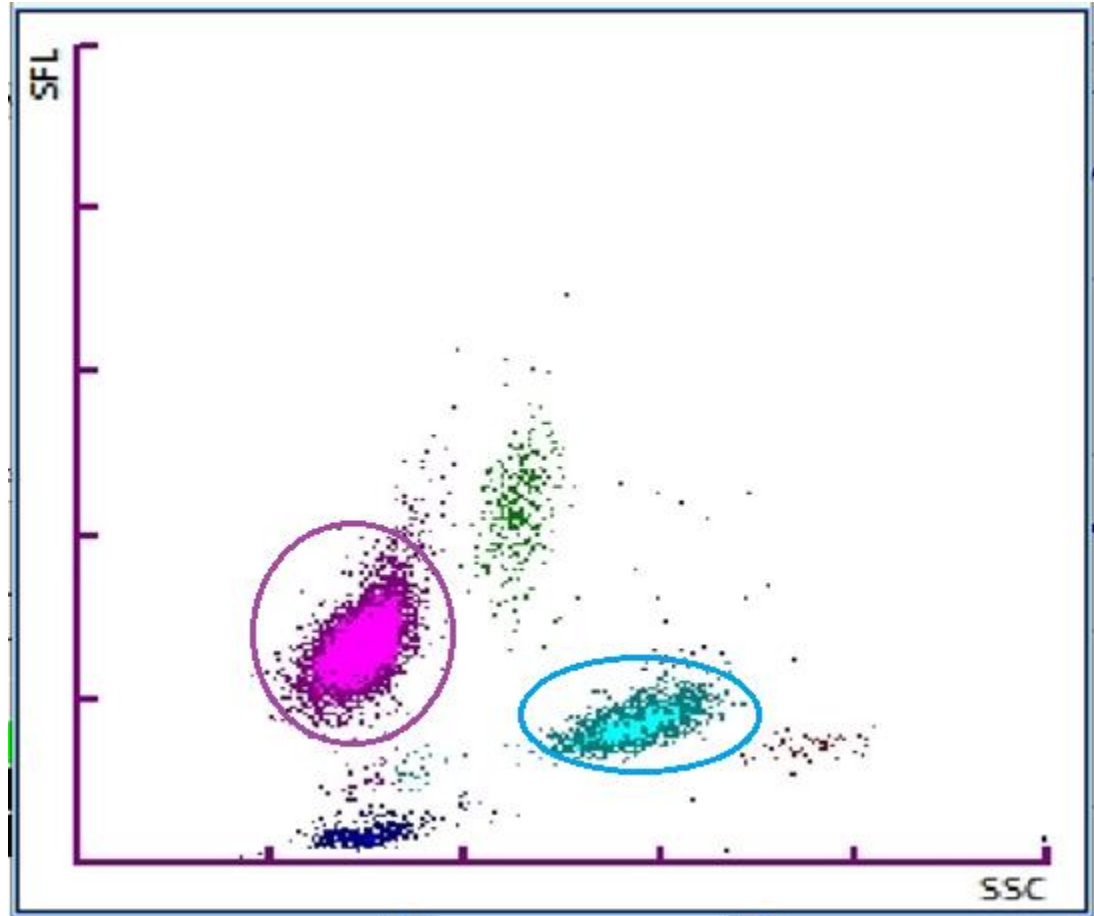
Example 3 Relative mild lymphocytosis



- **Neutrophils 42%**
- **Lymphocytes 49%**
- **Monocytes 6%**
- **Eosinophils 1%**
- *Basophils 1%*

Note the intensity of the neutrophil and lymphocyte clusters

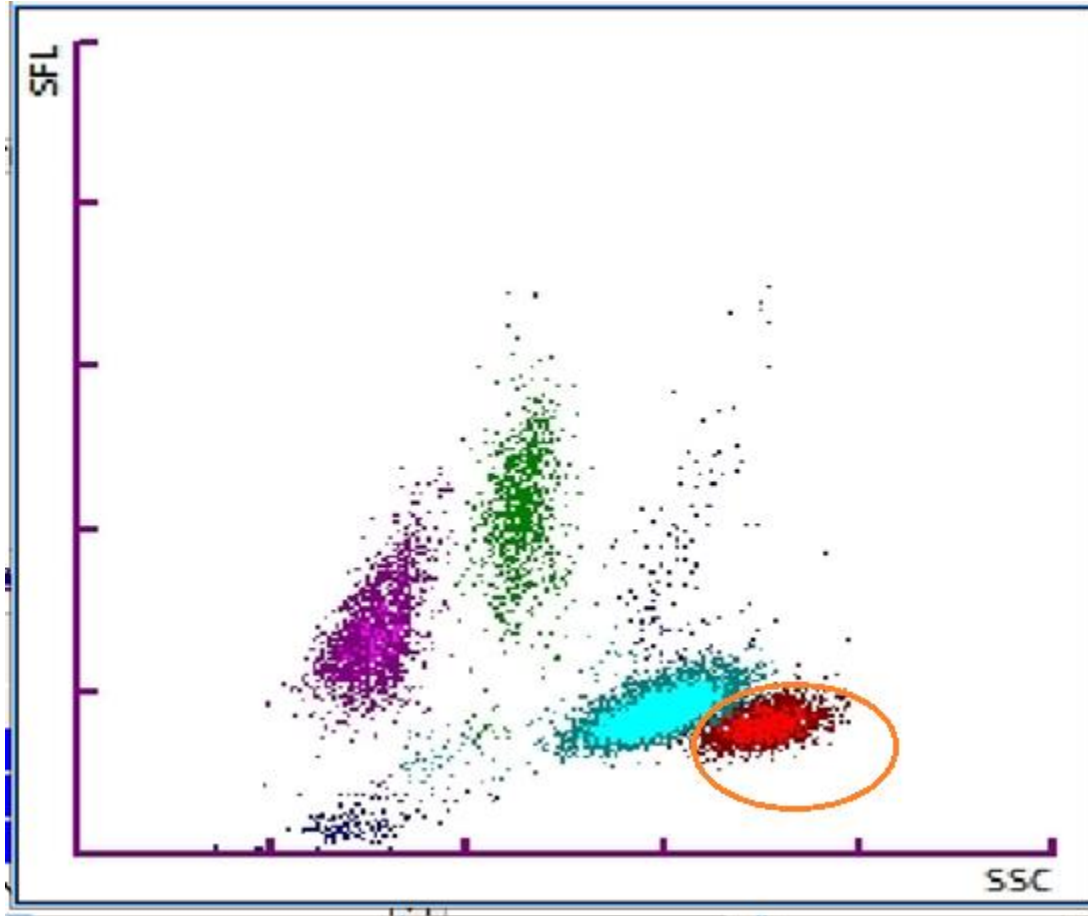
Example 4 Relative gross lymphocytosis



- **Neutrophils 19%**
- **Lymphocytes 76%**
- **Monocytes 3%**
- **Eosinophils 1%**
- *Basophils 1%*

Note the intensity of the neutrophil and lymphocyte clusters

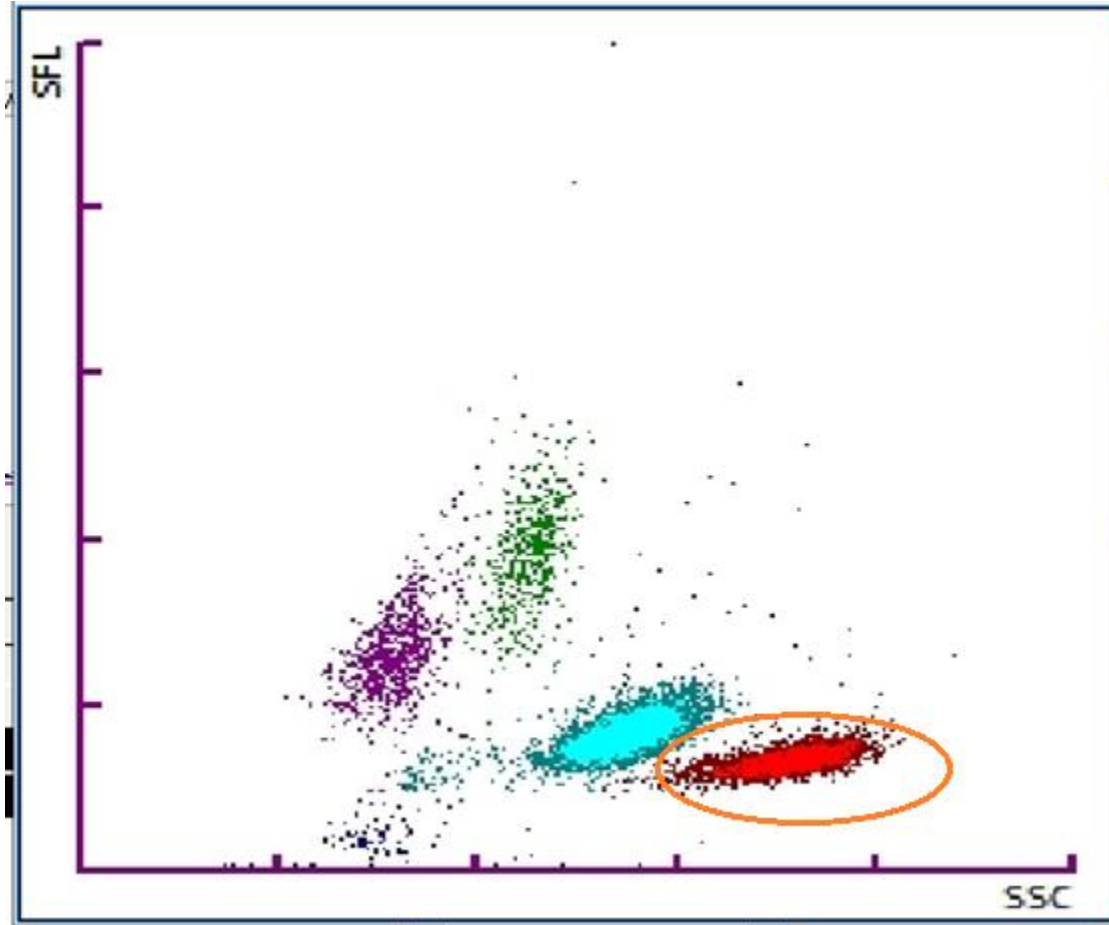
Example 5 Relative eosinophilia



- **Neutrophils 57%**
- **Lymphocytes 18%**
- **Monocytes 7%**
- **Eosinophils 17%**
- *Basophils 1%*

Note the intensity of the eosinophil cluster

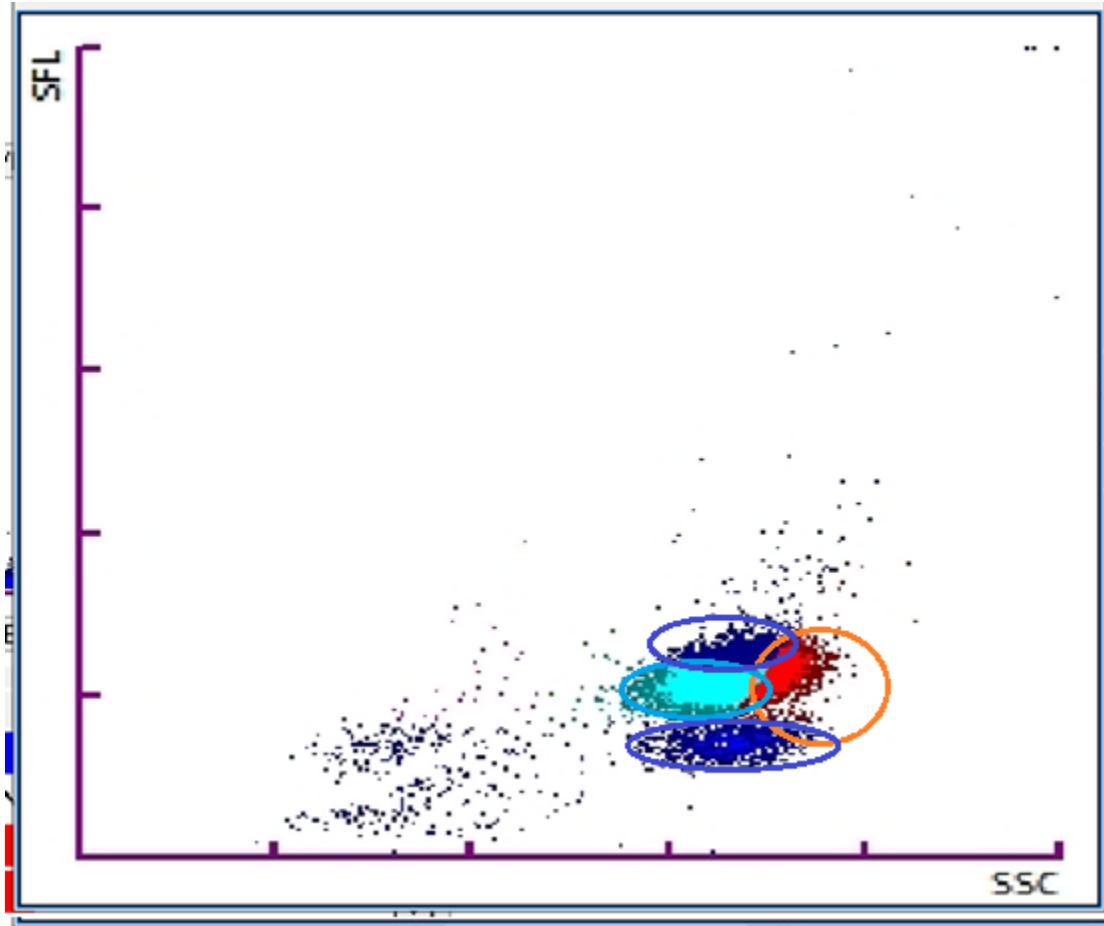
Example 5a Relative eosinophilia



- **Neutrophils 56%**
- **Lymphocytes 8%**
- **Monocytes 5%**
- **Eosinophils 30%**
- *Basophils 1%*

Note the intensity of the eosinophil cluster

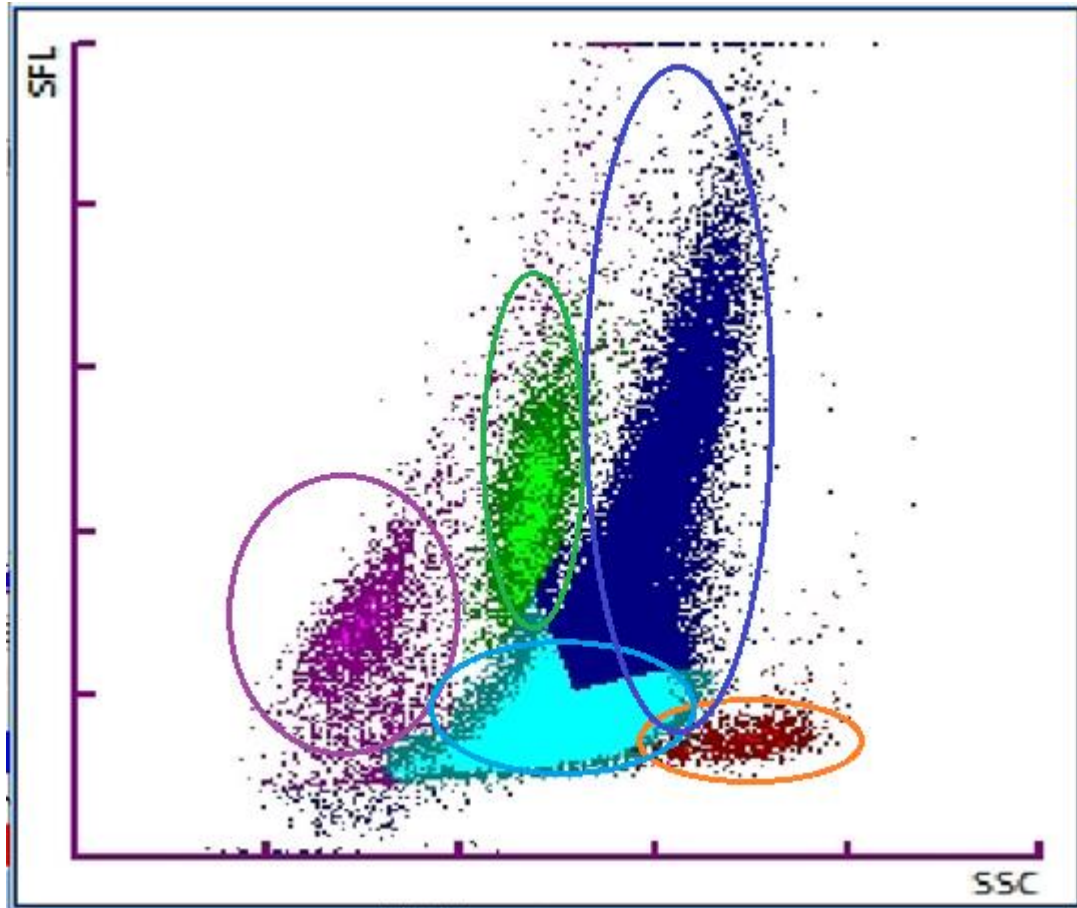
Example 6 QC sample scatterplot gating



- **Neutrophils 75%**
- **Lymphocytes 11%**
- **Monocytes 1%**
- **Eosinophils 19%**
- *Basophils 5%*

Note the shape of the cluster gating

Example 7 Immature Myeloid cells

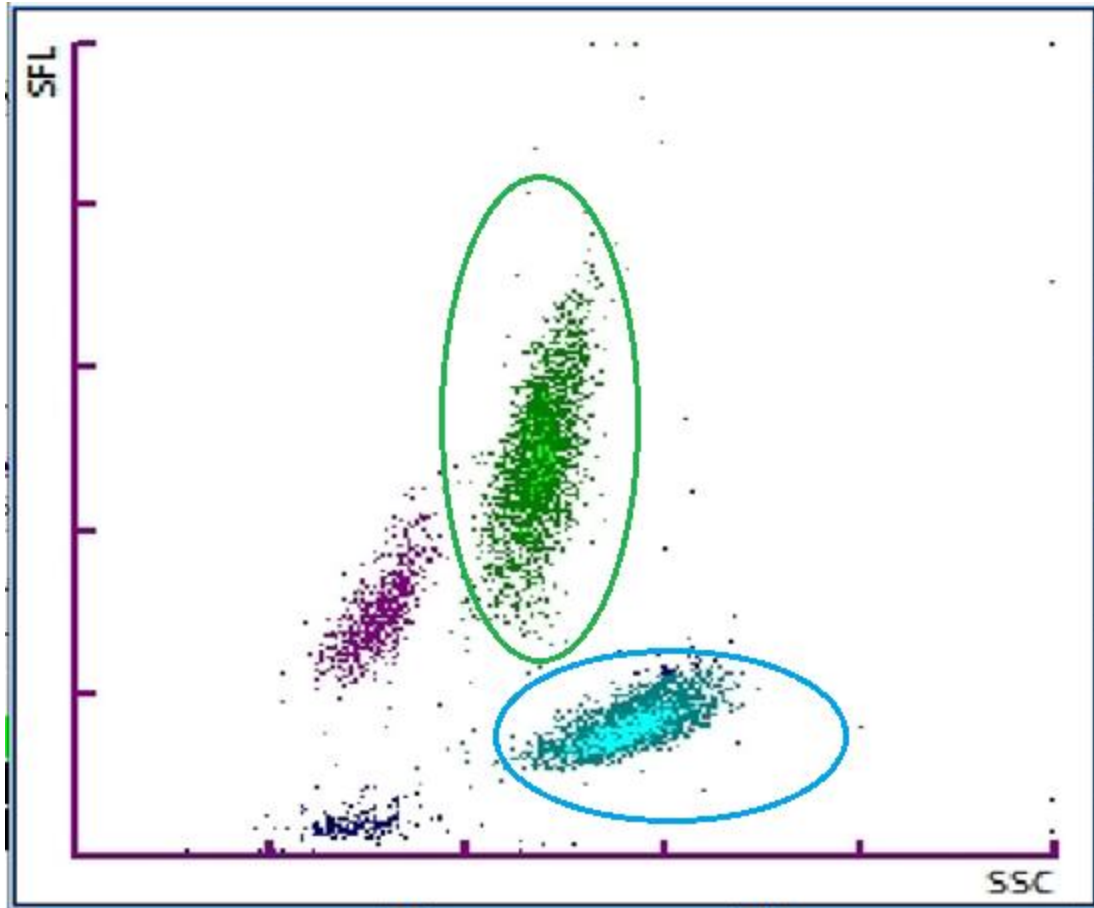


- **Neutrophils 91%**
- **Lymphocytes 3%**
- **Monocytes 5%**
- **Eosinophils 1%**
- *Basophils 1%*
- IG% 30

Note the shape and gating of the neutrophil and immature granulocyte clusters.

Note the relative intensity of the lymphocyte and monocyte clusters.

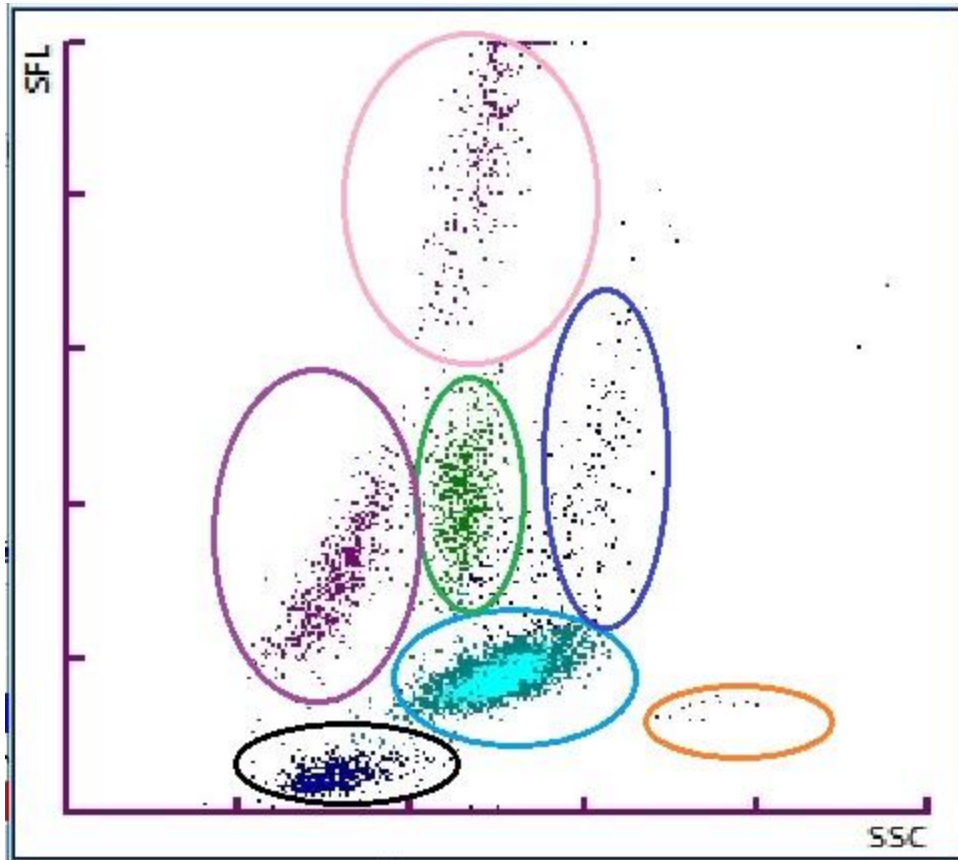
Example 8 Relative monocytosis



- **Neutrophils 49%**
- **Lymphocytes 11%**
- **Monocytes 40%**
- **Eosinophils 0%**
- *Basophils 0%*

Note the relative intensity of the neutrophil and monocyte clusters.

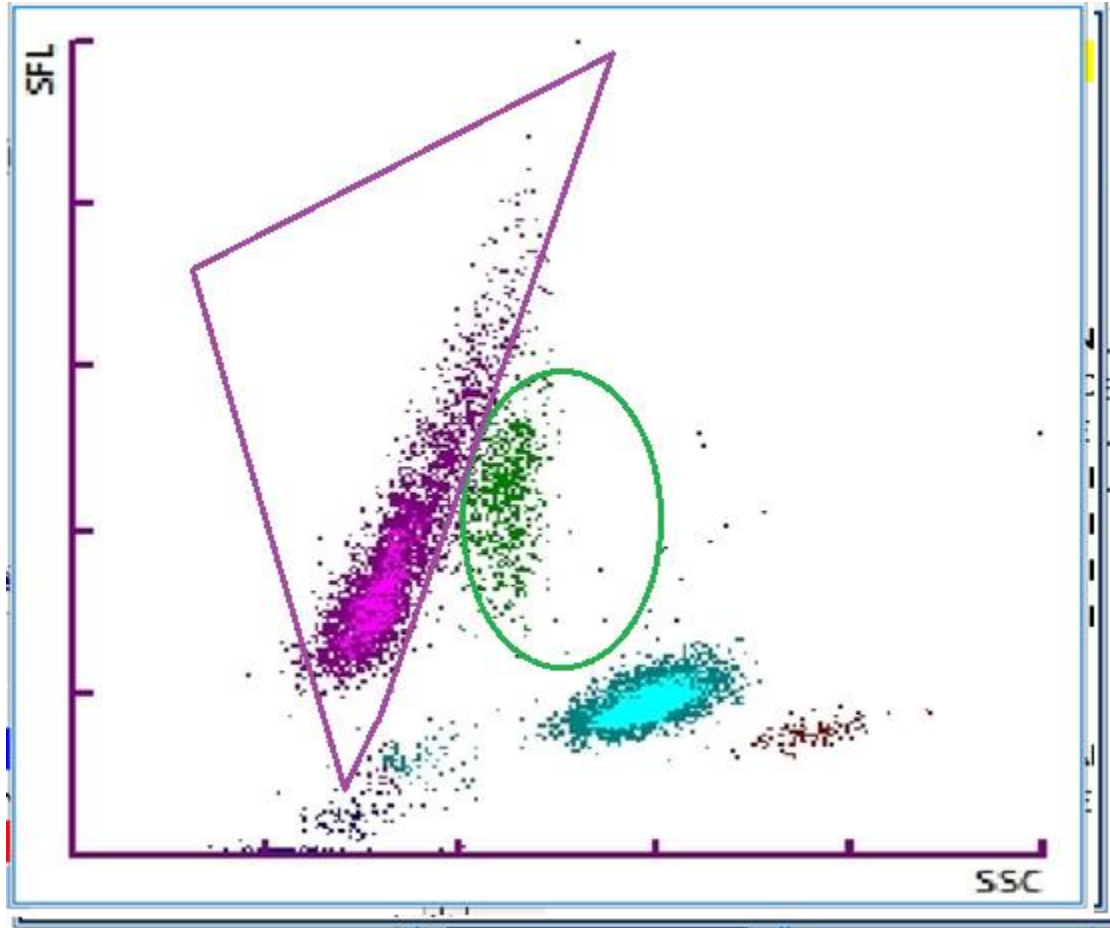
Example 9 Relative lymphocytes and immature granulocytes



- Neutrophils 73%
- Lymphocytes + reactive lymphocytes 16%
- Monocytes 10%
- Eosinophils 1%
- Immature myeloid cells 3%
- Junk

Note the reactive lymphocyte and immature granulocyte clusters

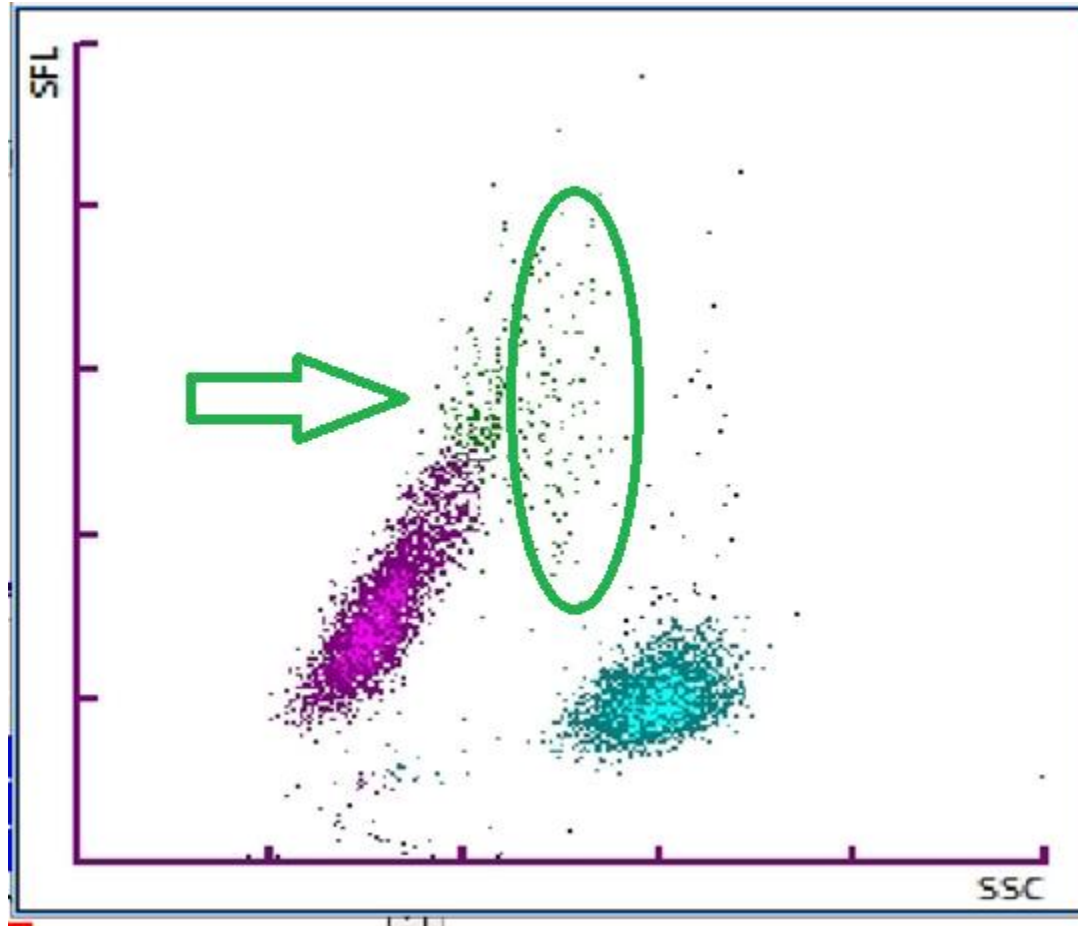
Example 10 Pleomorphic lymphocytosis



- **Neutrophils 46%**
- **Lymphocytes 48%**
- **Monocytes 5%**
- **Eosinophils 1%**
- *Basophils 0%*

Note the spread of the lymphocyte cluster but clear separation from the monocyte cluster

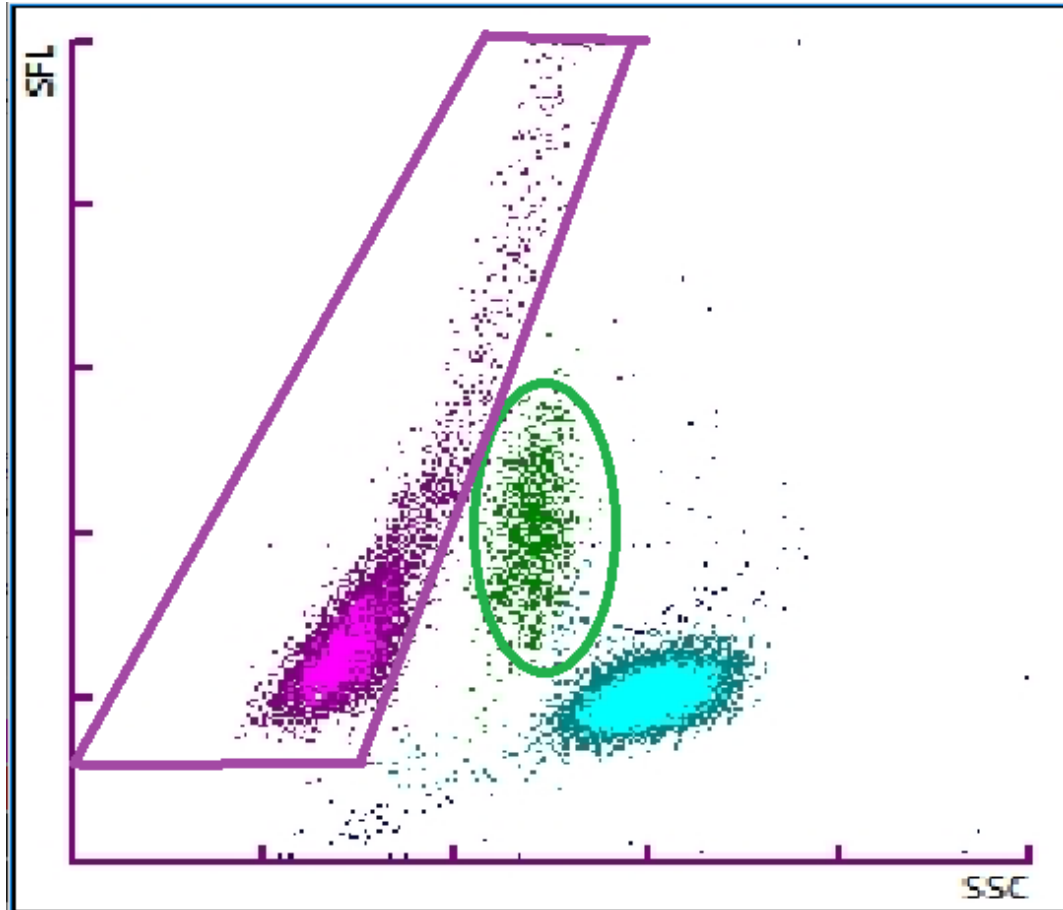
Example 11 Pleomorphic lymphocytosis with false monocytosis



- **Neutrophils 40%**
- **Lymphocytes 46%**
- **Monocytes 12% (Manual diff 2%)**
- **Eosinophils 1%**
- *Basophils 1%*

Note the spread of the lymphocyte cluster which continues into the green monocyte cluster

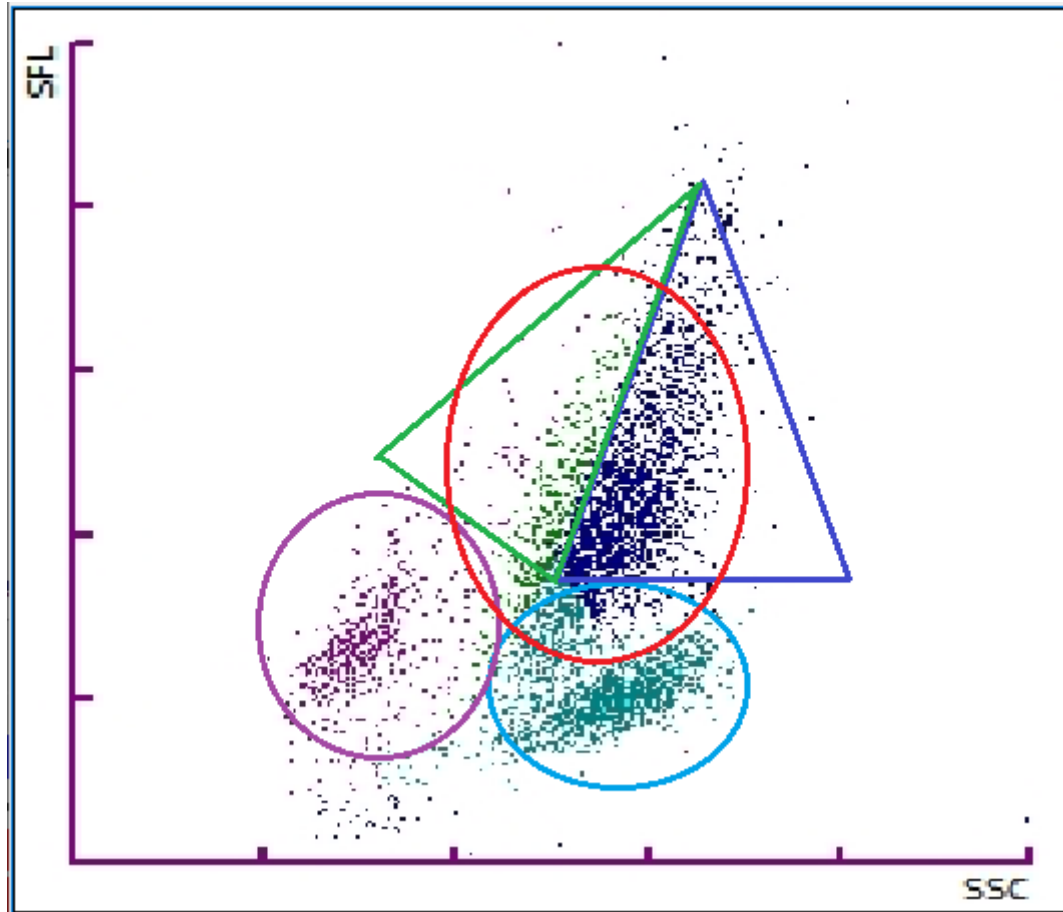
Example 12 Pleomorphic lymphocytosis clear monocyte distinction



- **Neutrophils 57%**
- **Lymphocytes 36%**
- **Monocytes 7%**
- **Eosinophils 0%**
- *Basophils 0%*

Note the spread of the lymphocyte cluster which continues into the green monocyte cluster

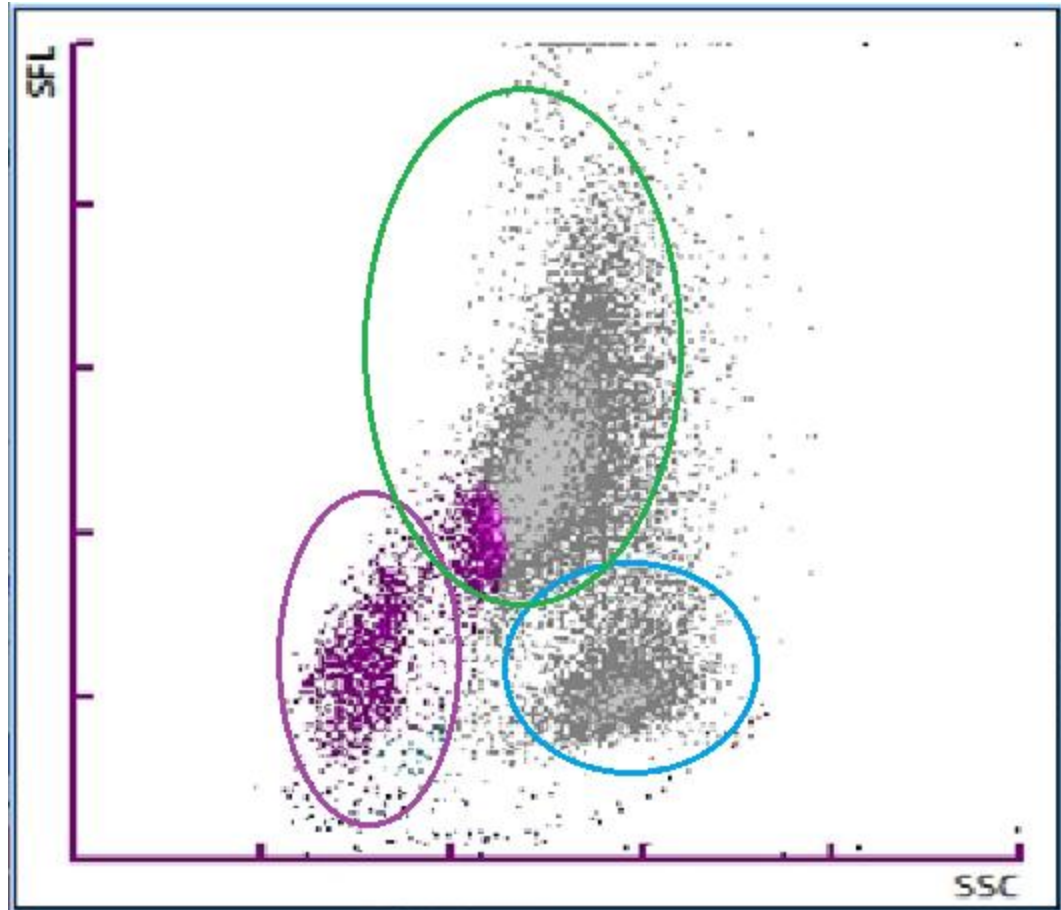
Example 13 Unreliable differentials



- **Neutrophils 80%**
- **Lymphocytes 12%**
- **Monocytes 8%**
- **Eosinophils 0%**
- *Basophils 0%*

Note the cluster that is classified as monocytes, IG's and neutrophils. This is a false diff in and APML

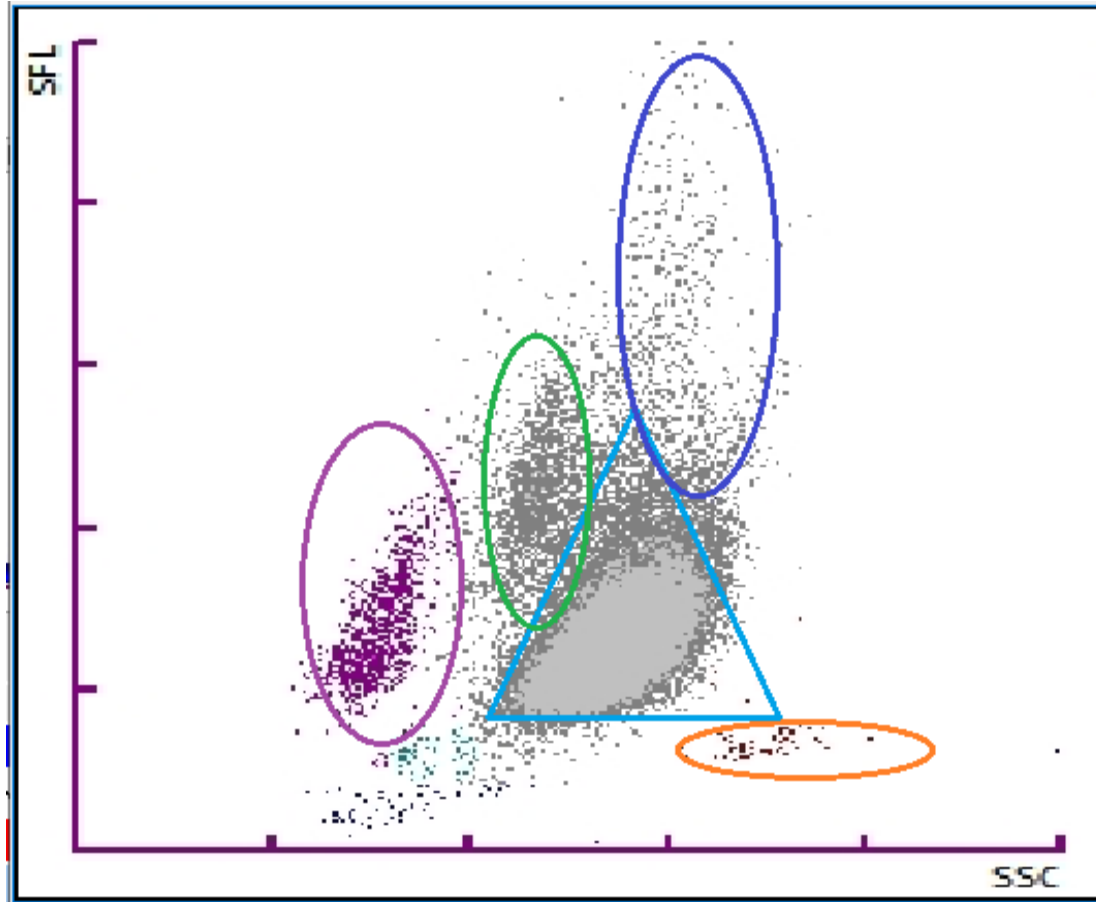
Example 14 Unreliable differentials



- **Neutrophils 35%**
- **Lymphocytes 10%**
- **Monocytes 54%**
- **Eosinophils 0%**
- *Basophils 0%*

Note the greyed out differential and the monocytes being counted as lymphocytes

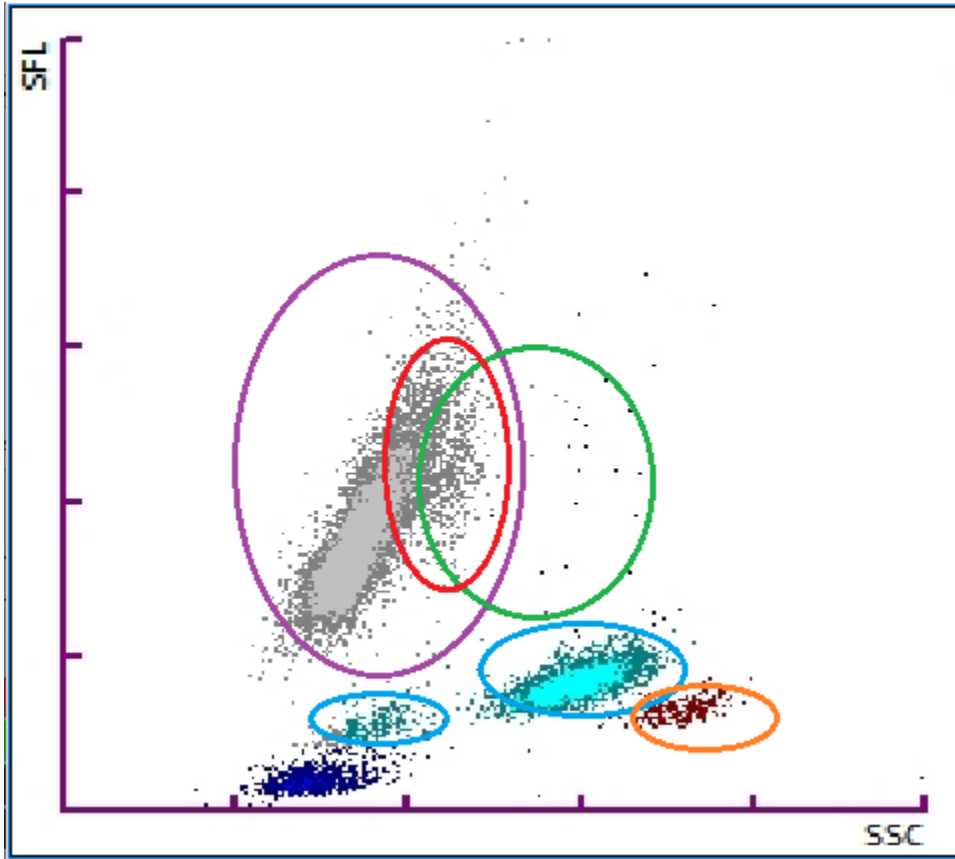
Example 15 Unreliable differentials



- **Neutrophils 91%**
- **Lymphocytes 2%**
- **Monocytes 6%**
- **Eosinophils 1%**
- *Basophils 0%*
- Immature myeloid cells present

Note the greyed out differential indicating analyser is not happy with the gating

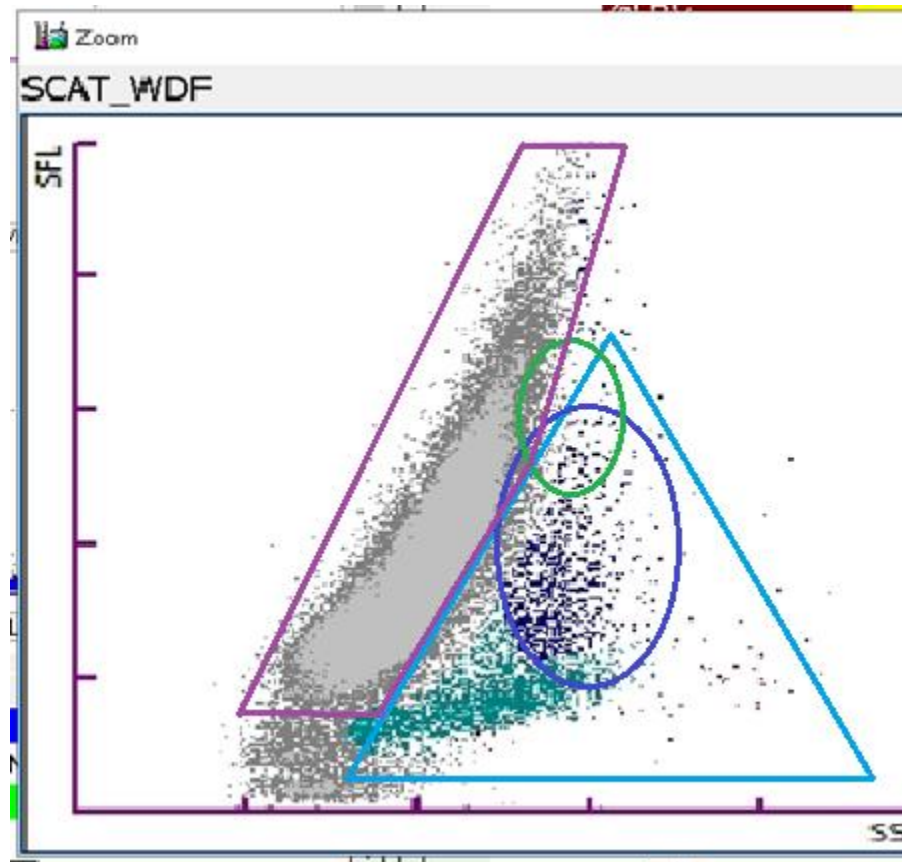
Example 16 Unreliable differentials



- **Neutrophils 20%**
- **Lymphocytes 66%**
- **Monocytes 11% (2% manual)**
- **Eosinophils 2%**
- *Basophils 1%*

Note the greyed out differential indicating analyser is not happy with the gating. Lymphocyte and monocyte overlap

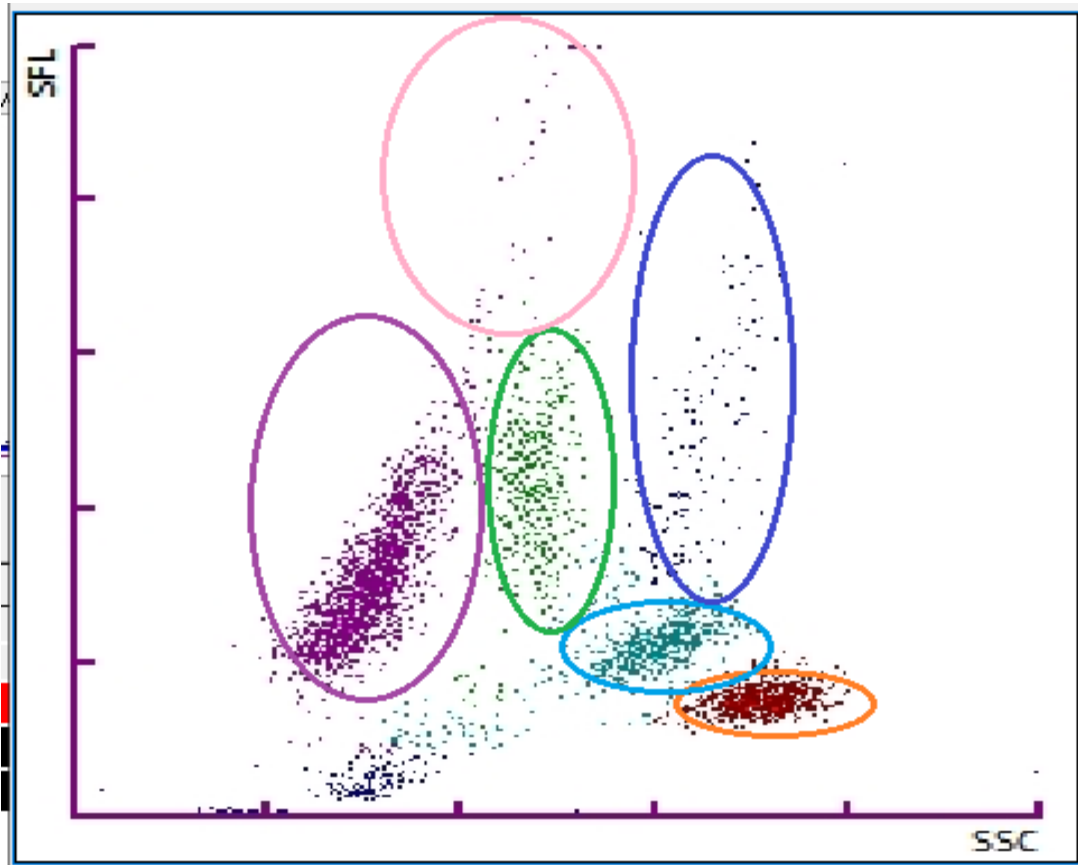
Example 17 Unreliable differentials



- **Neutrophils 3%**
- **Lymphocytes 32%**
- **Monocytes 65%**
- **Eosinophils 0%**
- *Basophils 0%*

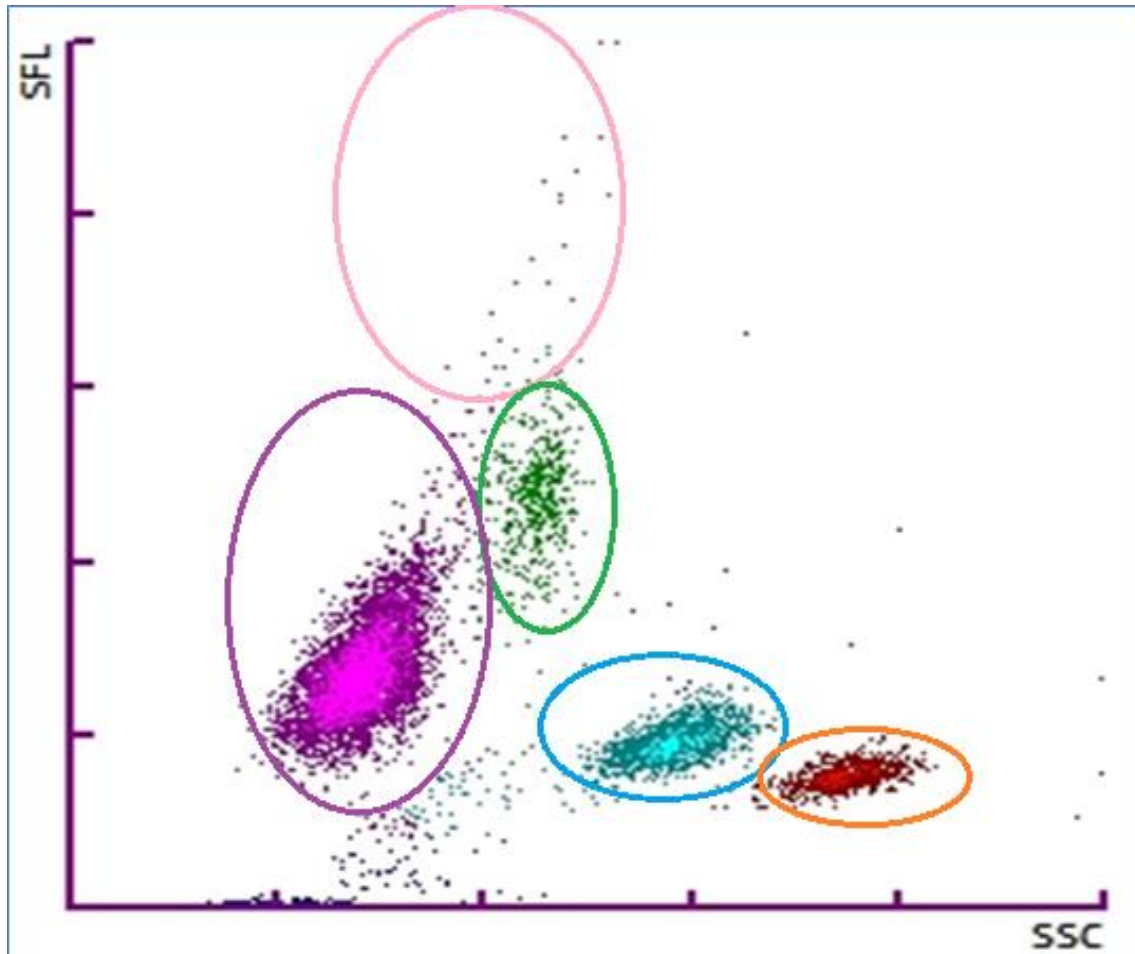
Note the greyed out differential indicating analyser is not happy with the gating. AML with majority of cells blasts and then neutrophils and left shift

Example review



- **Neutrophils 20%**
- **Lymphocytes 47%**
- **Reactive lymphocytes 1%**
- **Monocytes 11%**
- **Eosinophils 20%**
- ~~Basophils 1%~~
- **Immature granulocytes 3%**

Problem sample



- Impression from scatter
- Neutrophils – similar to eosinophils
- Lymphocytes - majority
- Reactive lymphocytes - minimal
- Monocytes – less than neut or eos
- Eosinophils – similar to neutrophils
- ~~Basophils 1%~~ - unable to say
- Immature granulocytes - minimal

Analyser differential

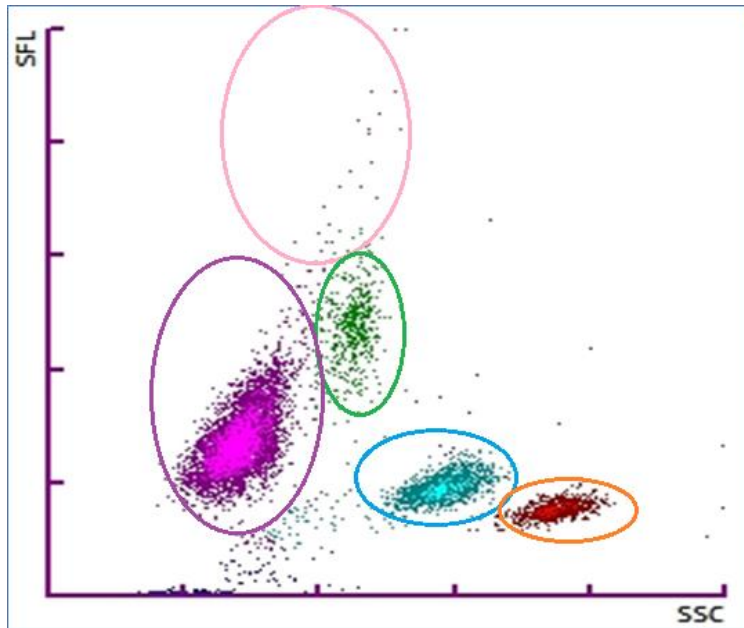
Neut 16.5
Lymph 68.2
Mono 5.9
Eos 9.1
Baso 0.3

Blood Film Review

- Cellular Spreading
- Blast cells and abnormal cells can preferentially spread along the edge or tail of the film.
- Manual differentials should always include a scan of the edge and tail to confirm **even** distribution of cells
- If spreading is uneven, incorporate the edge and tail into the manual differential to give a representative differential, or, use the automated differential if possible.

- Smear cells
- Smear cells may represent abnormal lymphocytes, blast cells or neutrophils.
- An albumin film can protect fragile cells for identification
- 4 Drops blood plus 1 drop 30% albumin
- An albumin film can help in the counting of a manual differential.
- Cells don't smear inside the analyser so the analyser auto differential can be valid when the film smear is not

Problem sample



Analyser differential

Neutrophils 16.5

Lymphocytes 68.2

Monocytes 5.9

Eosinophils 9.1

Basophils 0.3

Primary Result	Analyser%	Analyser#	Manual% Reported	Manual# Reported
Neutrophils	16.5	1.42	4	0.35
Lymphocytes	68.2	5.89	89	7.69
Monocytes	5.9	0.51	2	0.17
Eosinophils	9.1	0.79	5	0.43
Basophils	0.3	0.03	0	0.00

Retrospective Review	Analyser%	Primary film	Albumin slide Centre	Albumin side edge	Albumin slide centre and edge
Neutrophils	16.5	33	2	24	13
Lymphocytes	68.2	55	82	52	67
Monocytes	5.9	3	6	10	8
Eosinophils	9.1	7	8	14	11
Basophils	0.3	2	2	0	1
		With smear cells seen			

Looking at the scatter and retrospective table.
Which differential(s) would you report?