ICSH Differential Project

Progress Update December 2019

Sèvres, France

JCTLM meeting

Marie C Béné, Nantes

On behalf of Brent Wood, Seattle

Intent

- Replace morphology (CLSI H20-A2) with flow cytometry as the reference method for performing nucleated cell identification in blood

Intended Use

- Reference method for the validation of new automated hematology instrumentation
  - Manufacturers
  - Validation of high precision clinical studies
  - Value assignment to controls

- **NOT Intended for routine clinical use to:**
  - Clarify problematic differential following morphologic evaluation
  - Replace morphologic smear examination
  - Replace current differential on automated hematology analyzers

Populations

Core	
Lymphocytes	●
Monocytes	●
Neutrophils	●
Immature myeloids	●
Eosinophils	●
Basophils	●
Blasts	●
nRBCs	●

Miscellaneous	
Lymphocytes	
B cells	●
T cells	●
NK cells	●
Reactive	
Cytotoxic	
Plasma cells	
Dendritic cells	
CD16+ Monocytes	
Mast cells	

ICSH 10/2007

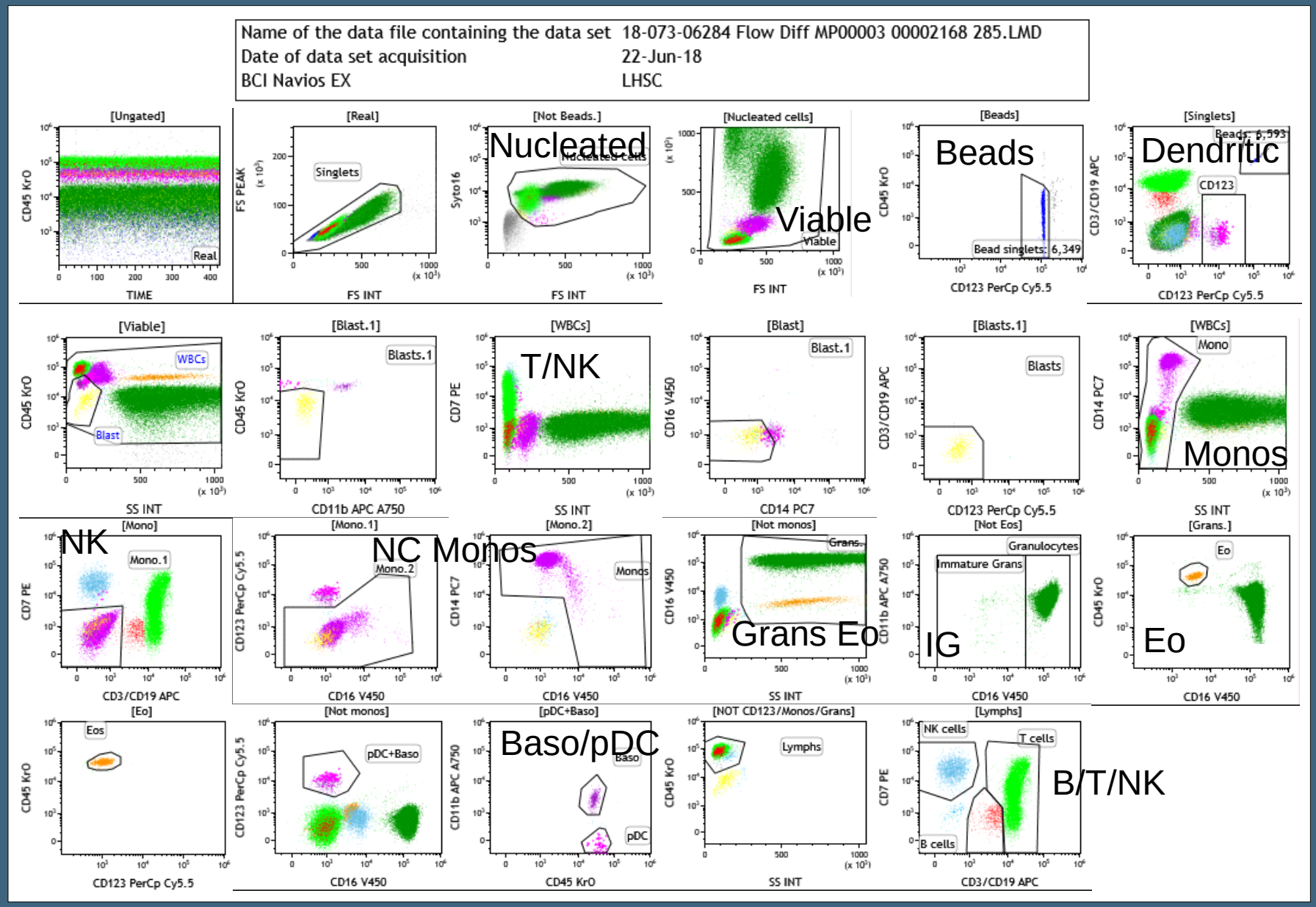
Reference WBC diff working project: Proposal for 2nd Phase Study

Panel of antibodies

Syto16	Nucleic acid binding dye
CD45-KO	Leukocytes
CD3-APC	T Lymphocytes
CD19-APC	B Lymphocytes
CD7-PE	NK cells
CD123-PerCP-Cy5.5	Basophils, pDendritic Cells
CD14-PE-Cy7	Monocytes
CD16-v450 or Pacific Blue	Neutrophils, immature myeloid cells, NK, monocyte subset
CD11b-APC-A750	Myeloid cells, monocytes, basophils, NK

- Lyse, no wash with counting beads
- Syto-16 provides **nucleated cell identification**
- 6 requested populations are defined by **positive markers**
- **Blasts are identified by exclusion**, since phenotypes are widely divergent
- Nucleated RBCs with no positive antigens, but are identifiable
- **Redundancy**: most cells are defined by multiple markers
- *Secondary objective: additional cell type would be detectable if abnormal*

Normal Example



ICSH WBC differential working project
Validation Protocol

Validation performed in Melbourne and Seattle, including:

Sensitivity – 3 samples by serial dilution in triplicate
Specificity – 3 samples vs. H20-A2
Reproducibility
Within run – 3 samples in triplicate
Between run – Immunotrol over 5 days
Stability – 5 samples at 0, 24 and 48 hours
Linearity – 3 samples serially diluted 7 times run in triplicate

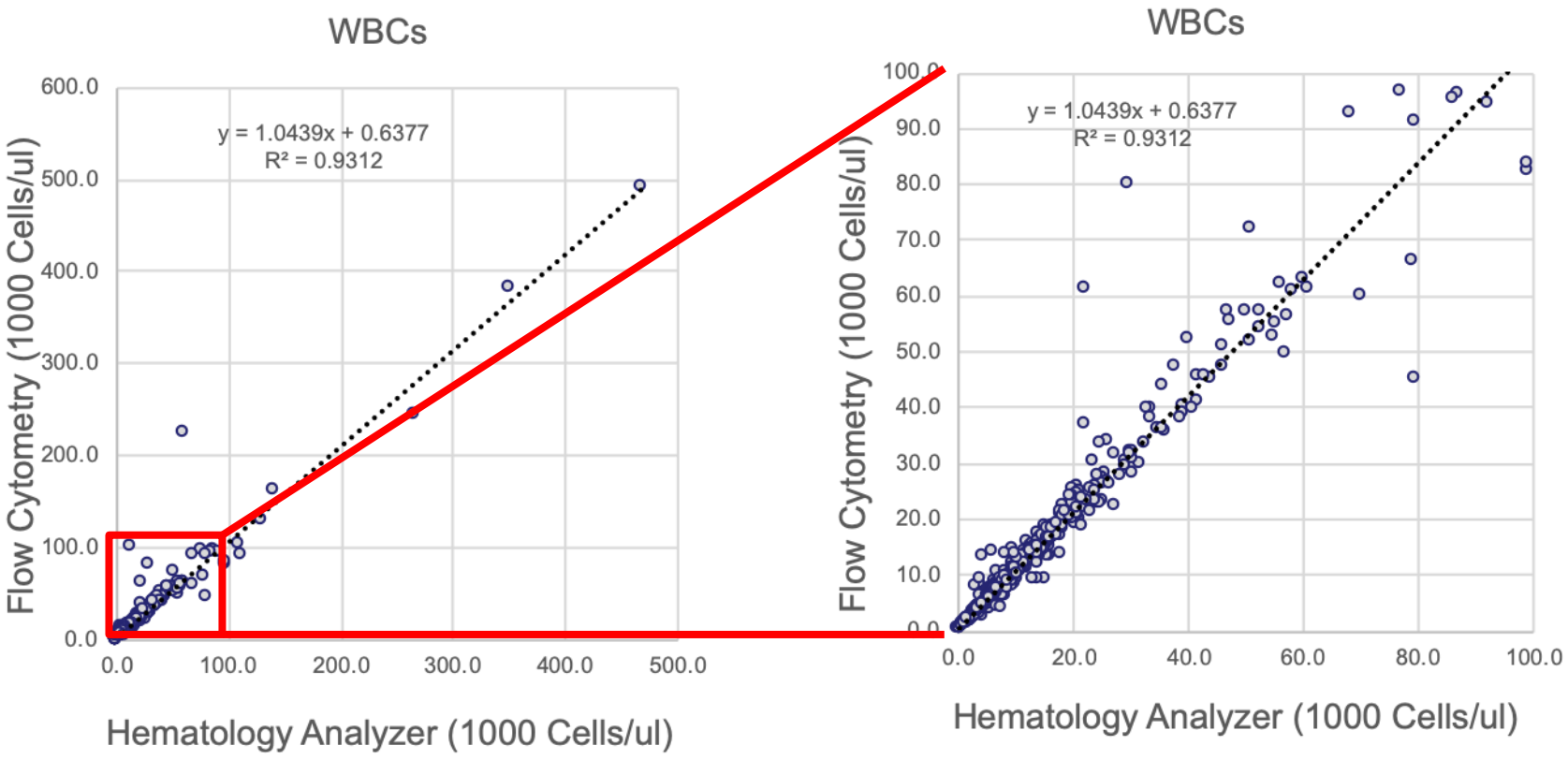
Accuracy and Robustness – 150 samples (30 normals) at 5 sites vs. H20-A2

London, Canada (Michael Keeney, Ben Hedley)	Completed
Nantes, France (Marie Christine Bene)	Incomplete
Melbourne, Australia (Peter Gambell)	Complete
Beijing, China (Chenxue Qu)	Complete
Seattle, USA (Brent Wood)	Complete

Accuracy

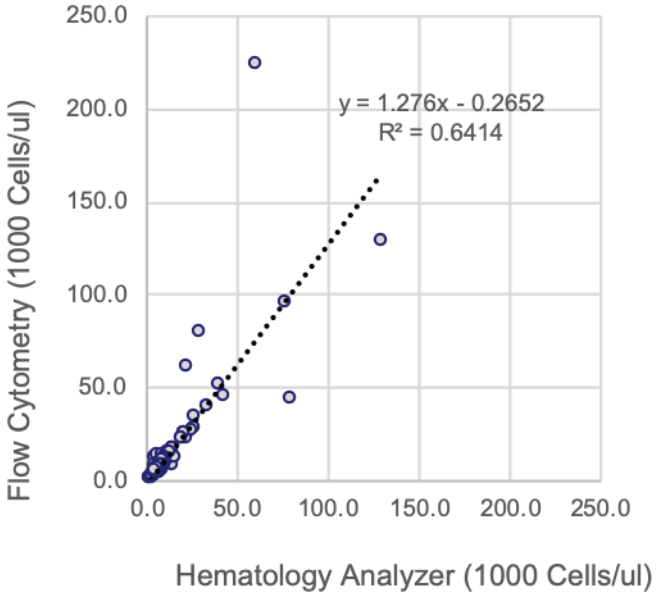
- Intent
  - Real world implementation
    - Reagents
    - Procedure (SOP)
    - Example of data analysis
- Reality
  - 4 of 5 sites executed procedure successfully
    - 1 site had technical difficulties with cytometer programming
  - Representative instrumentation
    - Hematology analyzers – BC, Sysmex, Abbott
    - Cytometers – BD, BC
  - Data submitted for central review
    - Variability in content

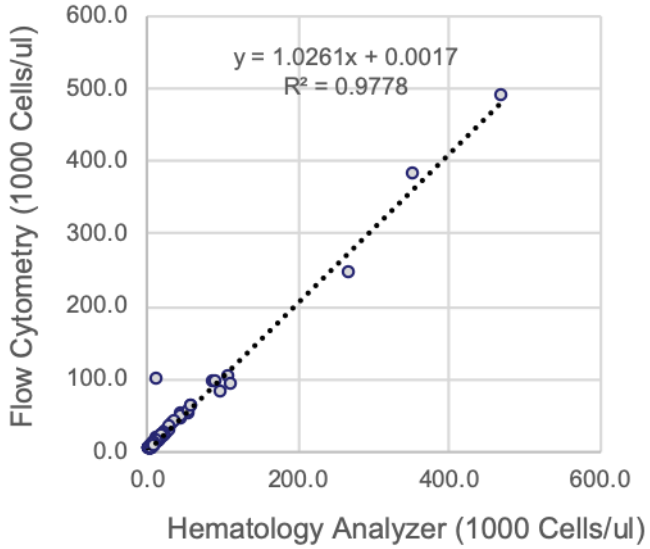
WBC Count

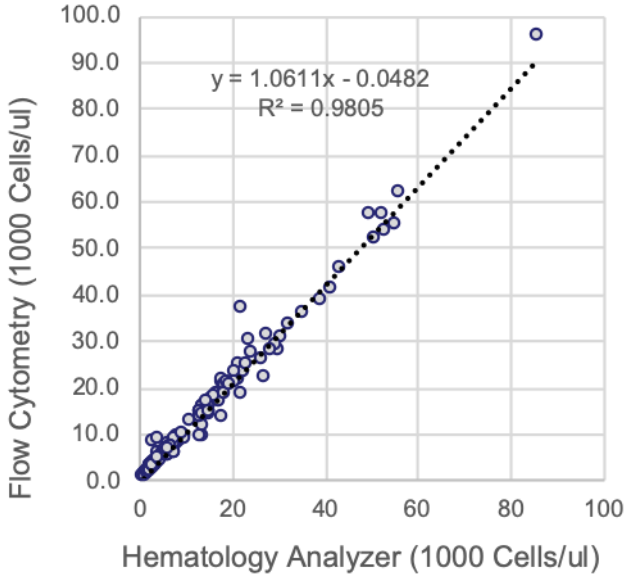


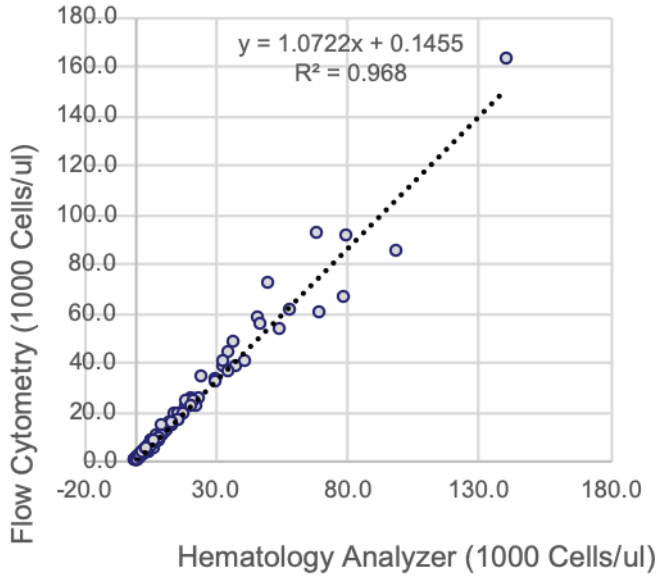
N = 616

WBC Count


y = 1.276x - 0.2652
R² = 0.6414
Flow Cytometry (1000 Cells/ul)
Hematology Analyzer (1000 Cells/ul)
0.0
50.0
100.0
150.0
200.0
250.0

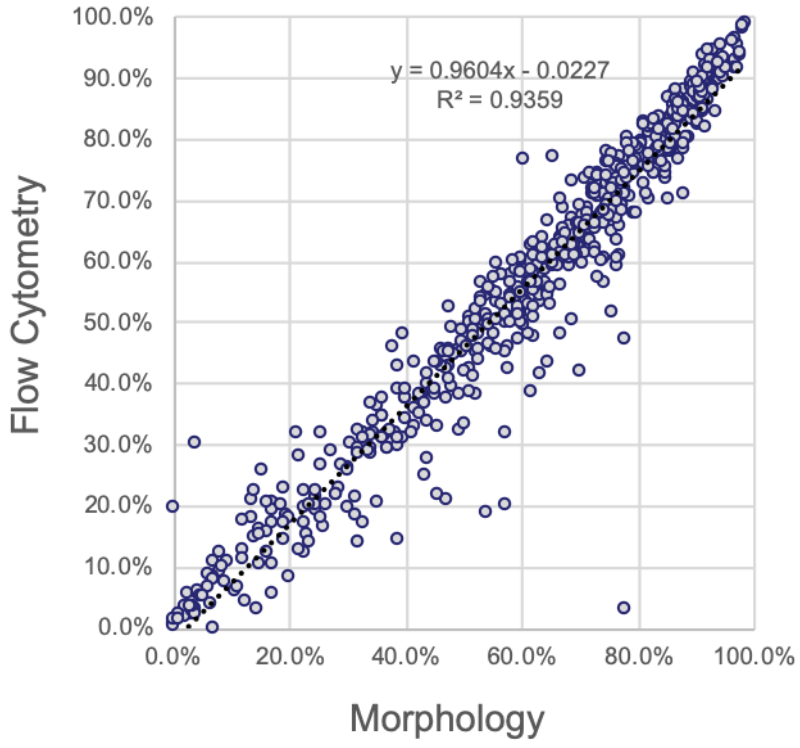

y = 1.0261x + 0.0017
R² = 0.9778
Flow Cytometry (1000 Cells/ul)
Hematology Analyzer (1000 Cells/ul)
0.0
100.0
200.0
300.0
400.0
500.0
600.0


y = 1.0611x - 0.0482
R² = 0.9805
Flow Cytometry (1000 Cells/ul)
Hematology Analyzer (1000 Cells/ul)
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
0
20
40
60
80
100

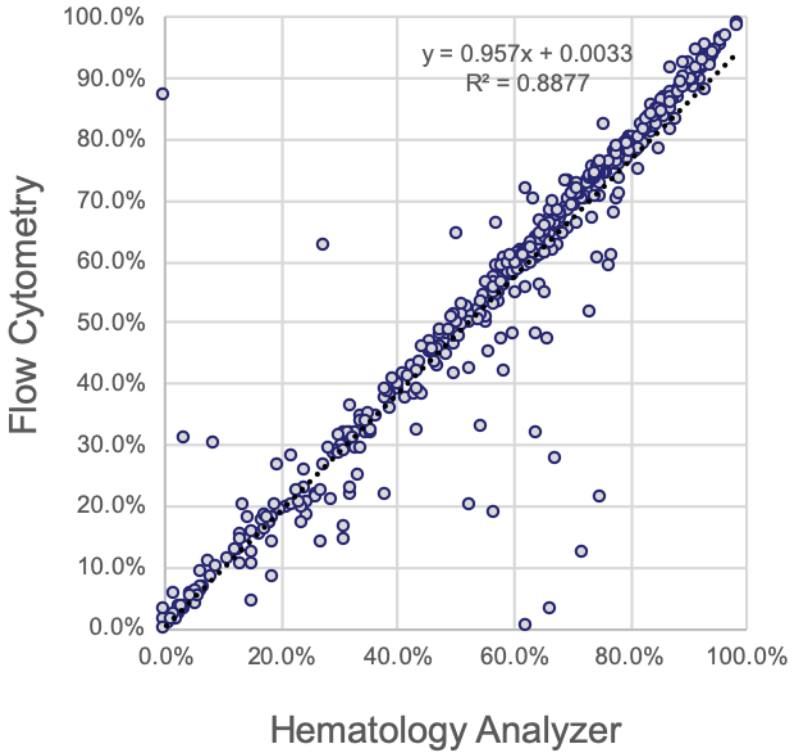

y = 1.0722x + 0.1455
R² = 0.968
Flow Cytometry (1000 Cells/ul)
Hematology Analyzer (1000 Cells/ul)
-20.0
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0
160.0
180.0
30.0
80.0
130.0

Neutrophil %

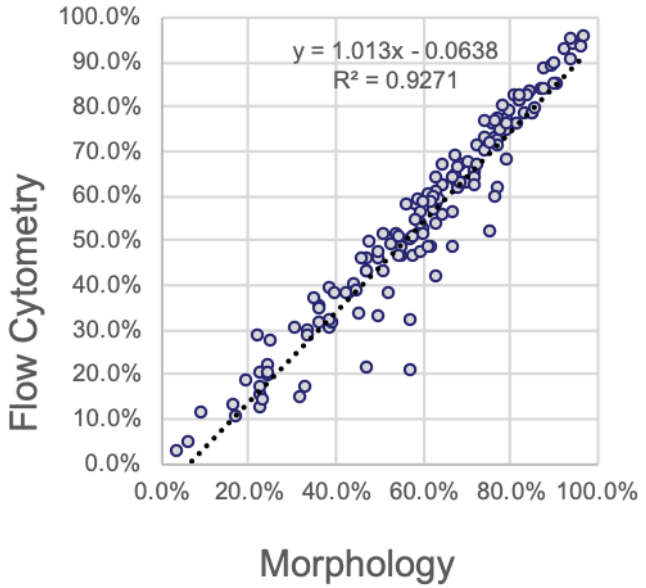
R² 0.93

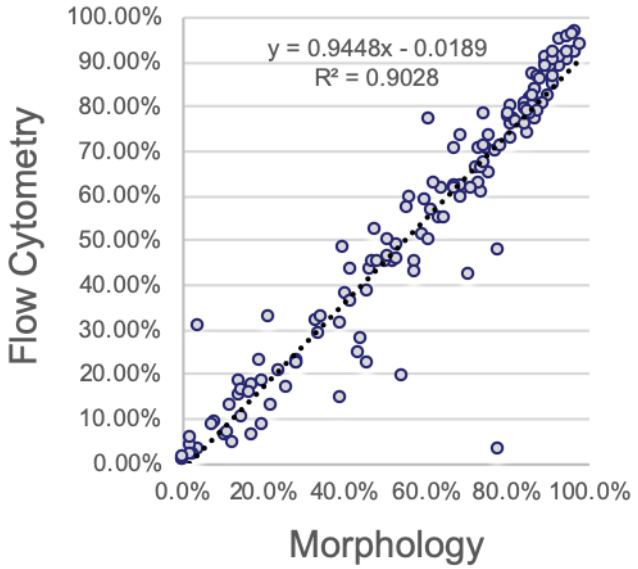


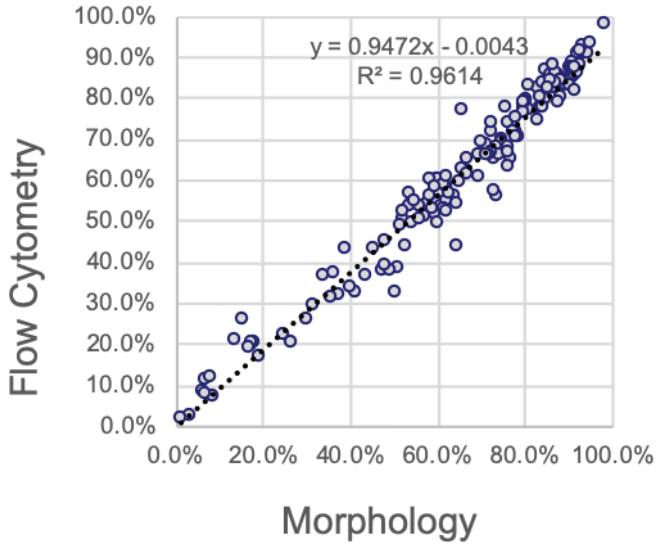
R² 0.89

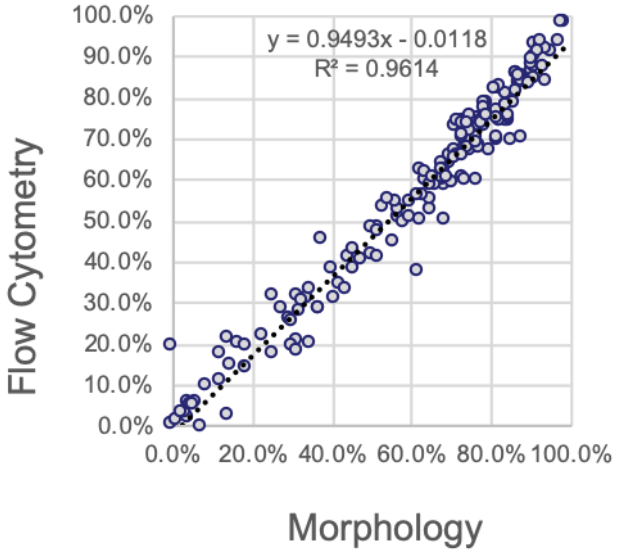


Neutrophils %

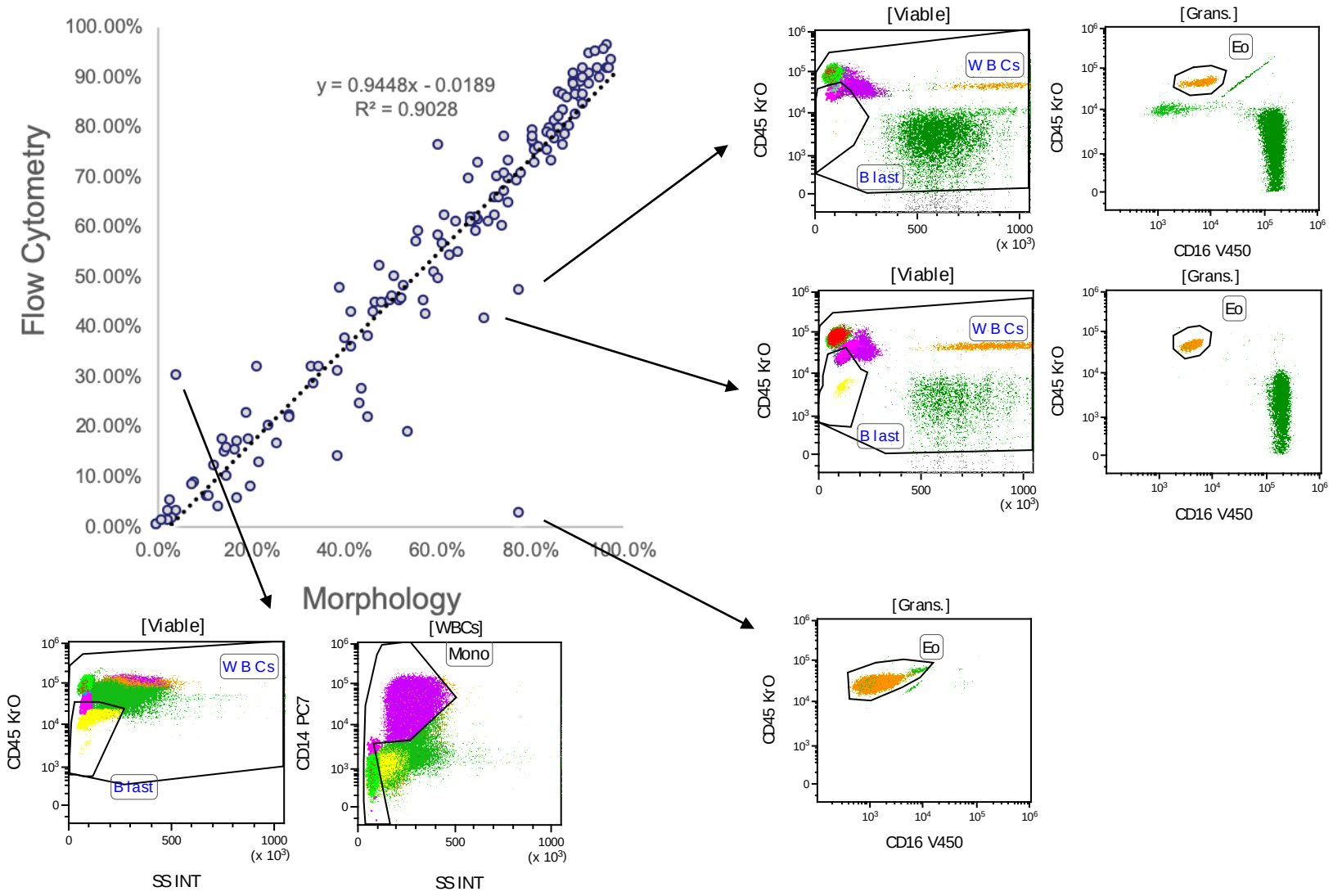




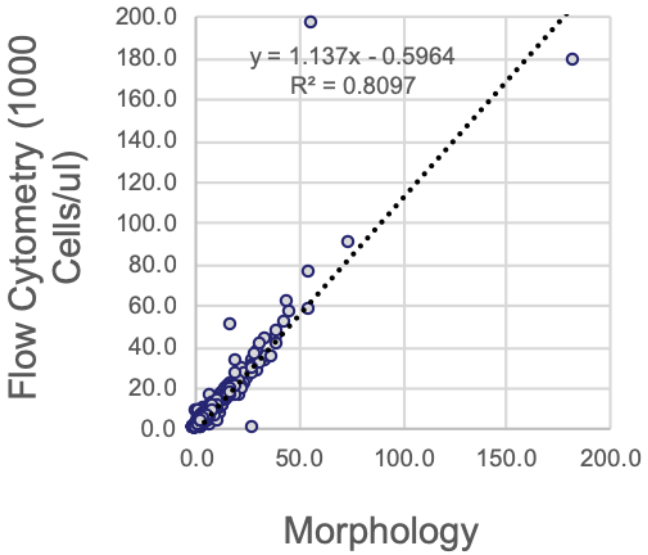


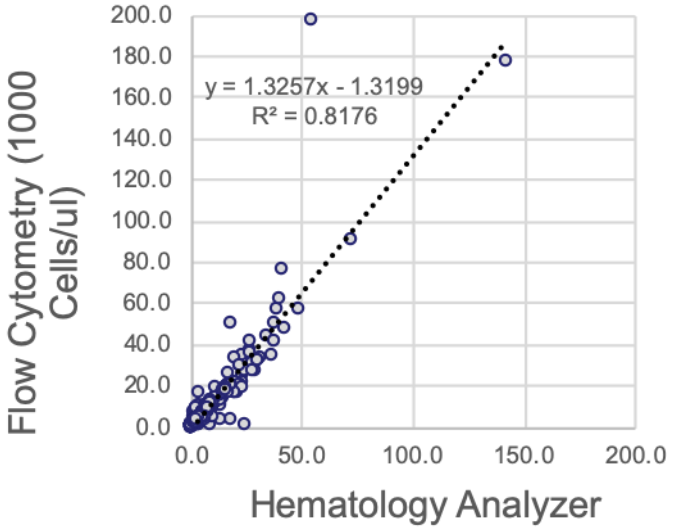


Neutrophils %

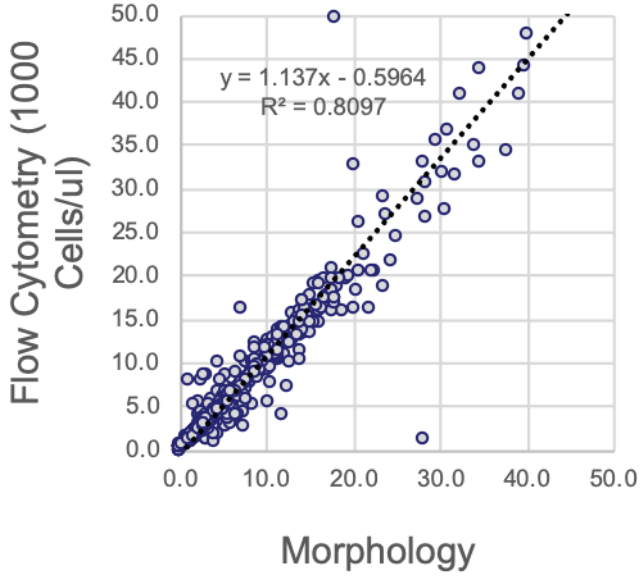


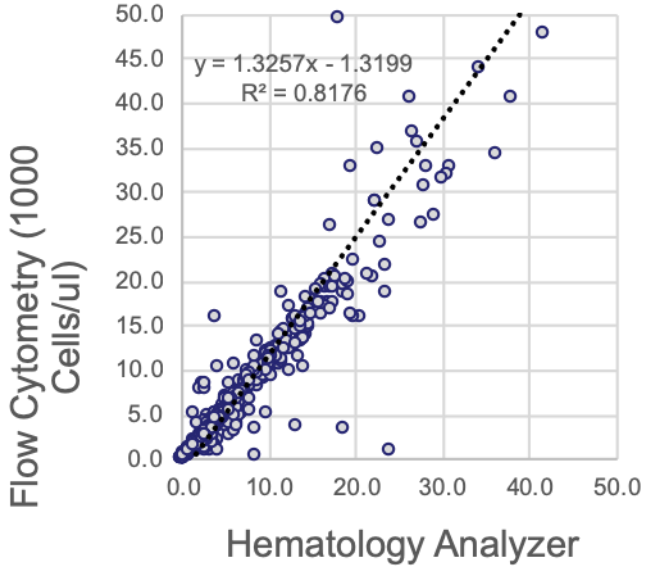
Neutrophils Absolute





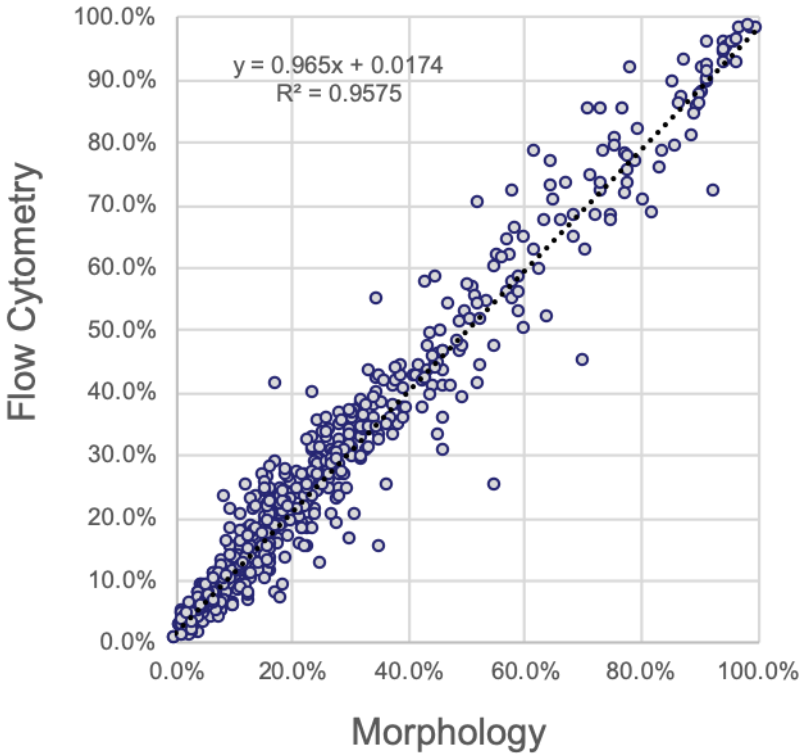
R² 0.81



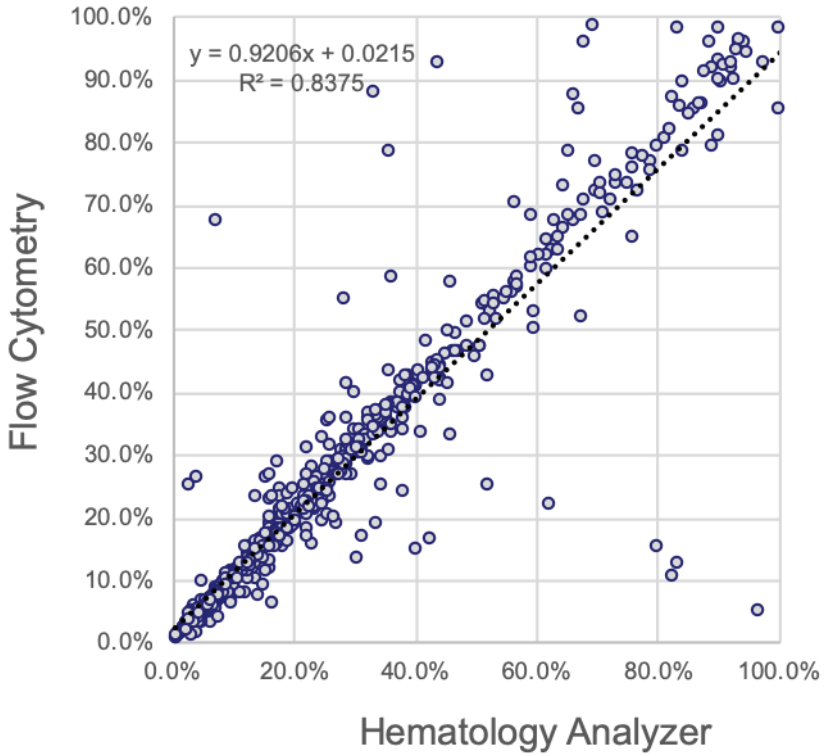


Lymphocytes %

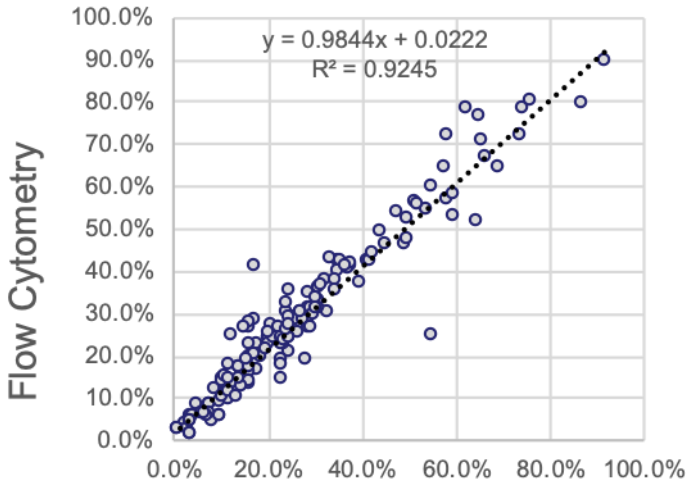
R^2 0.96

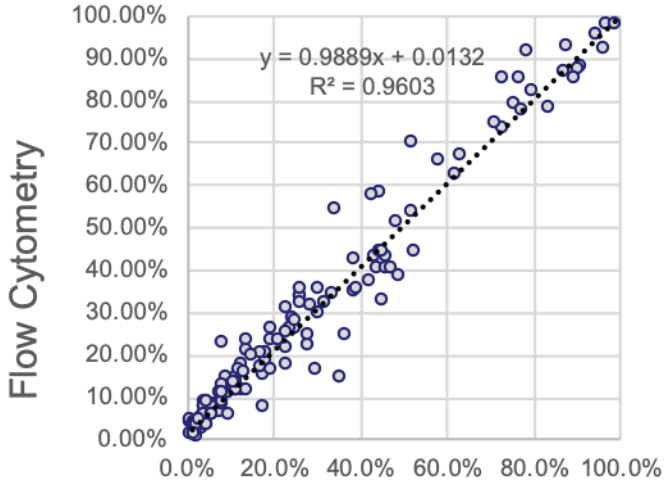


R^2 0.84

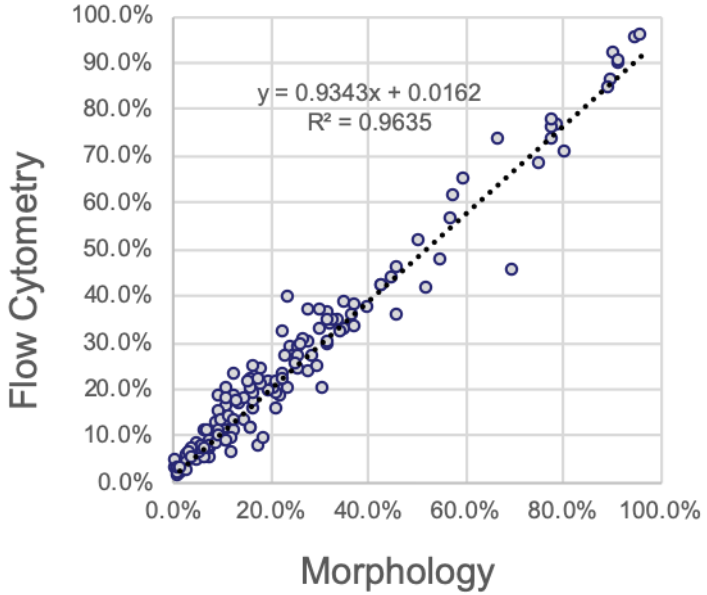


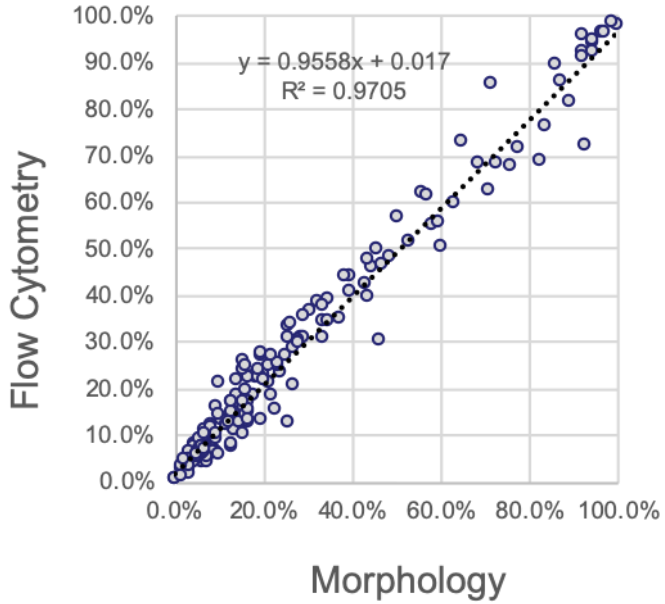
Lymphocytes %



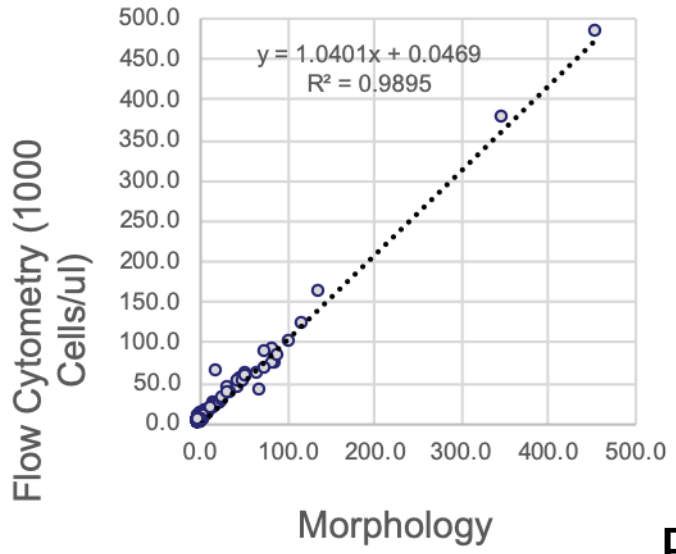


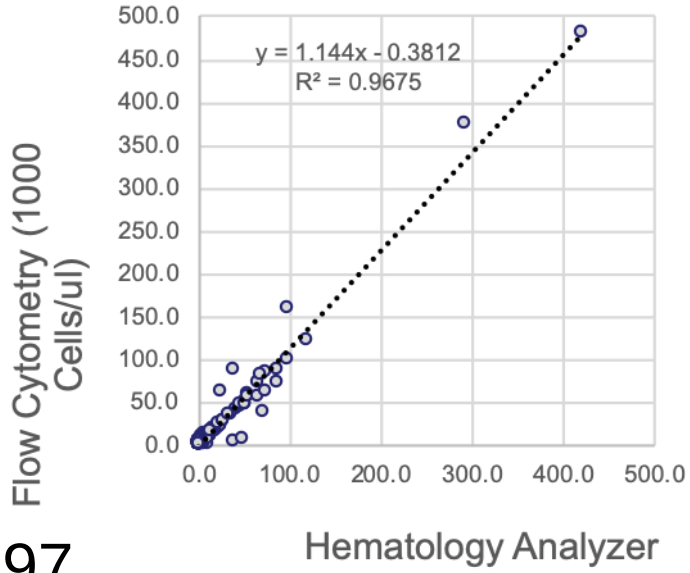
R² 0.93 - 0.96



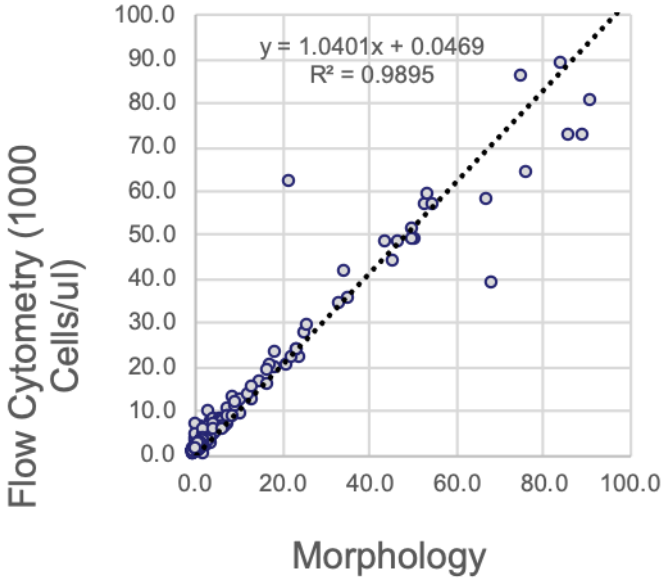


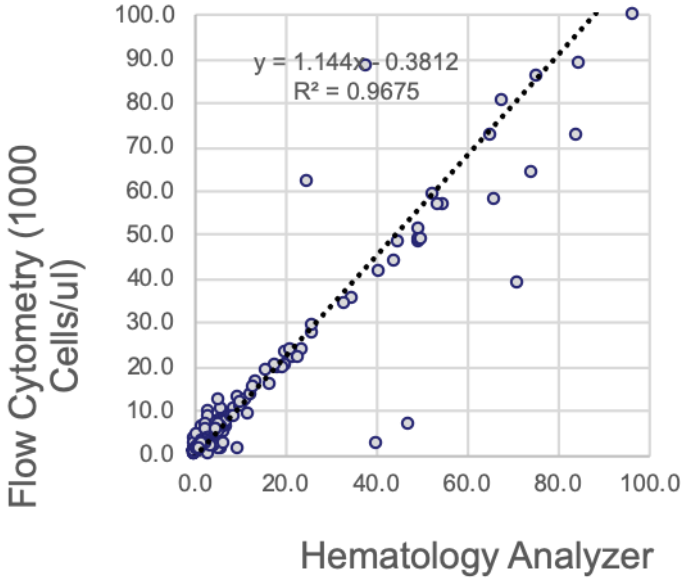
Lymphocytes Abs





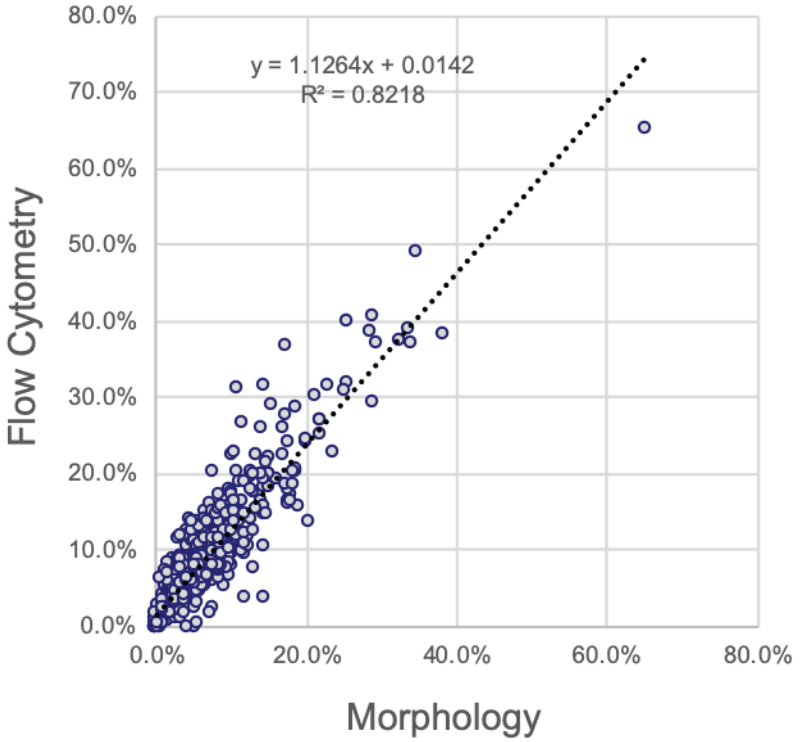
R² 0.97



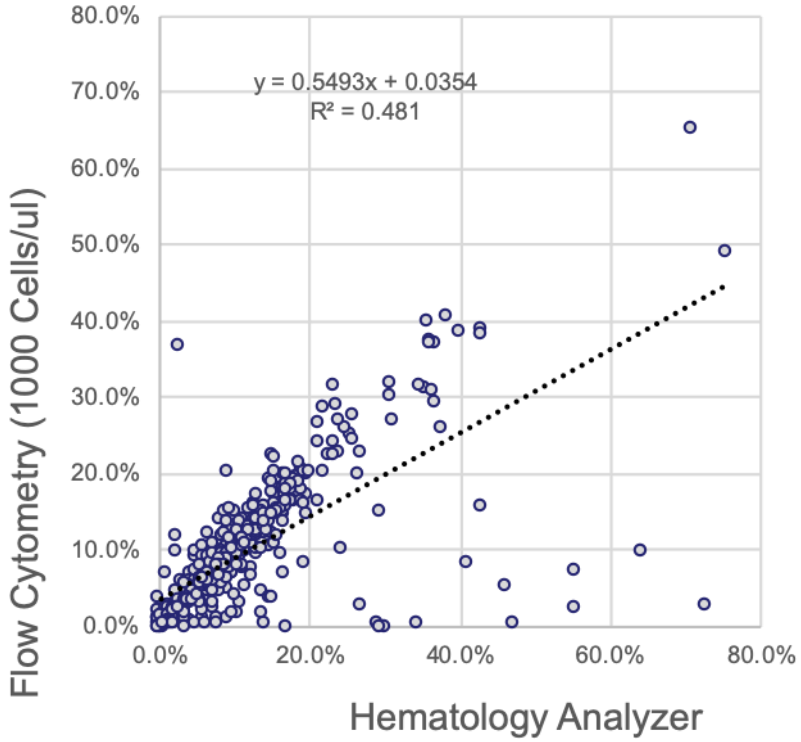


Monocytes %

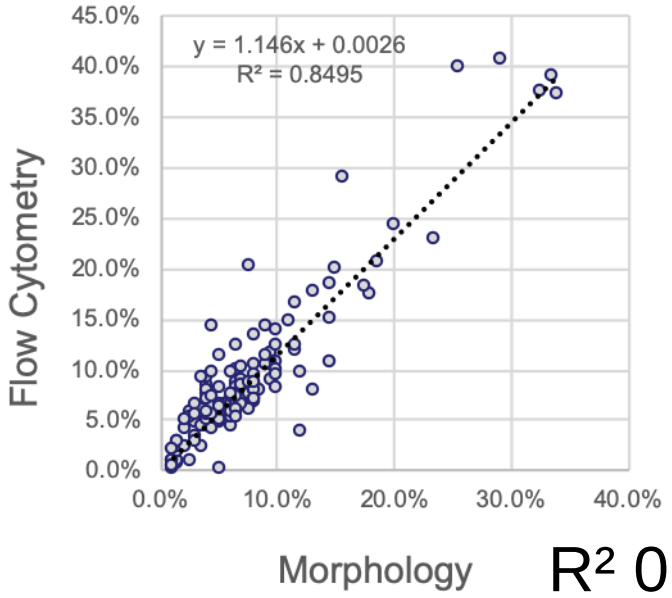
R^2 0.82

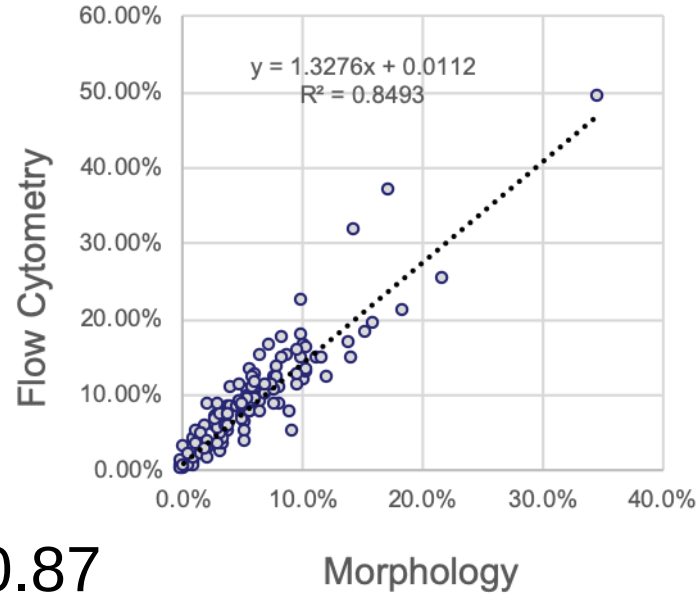


R^2 0.48

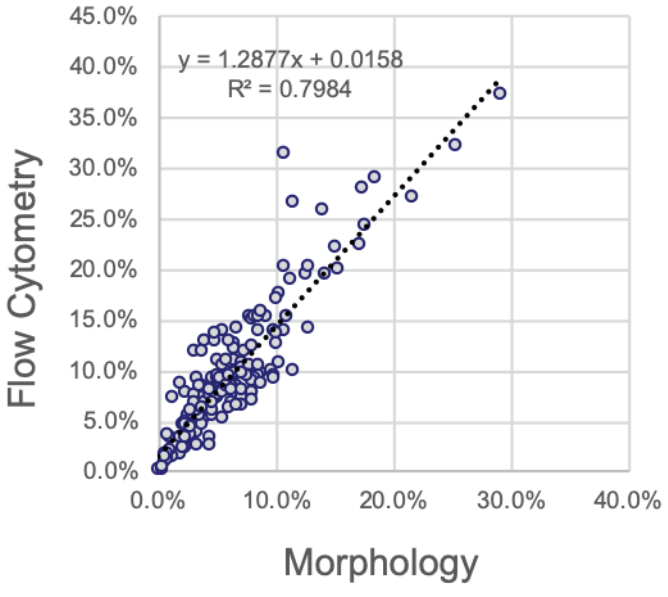


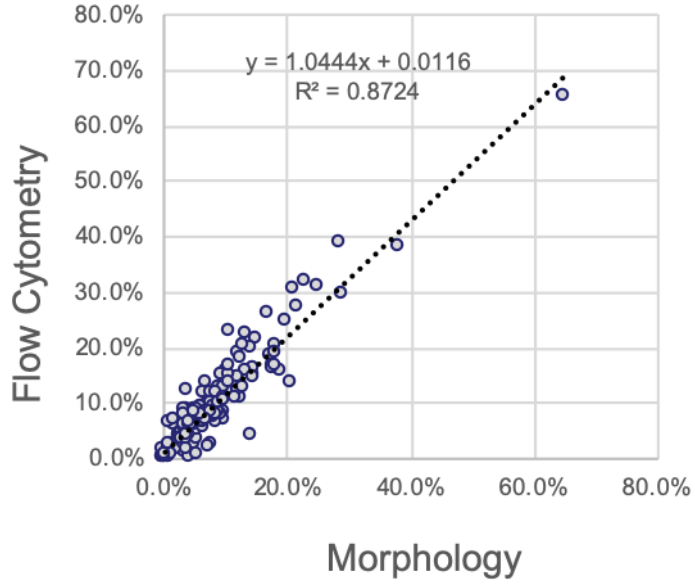
Monocytes %





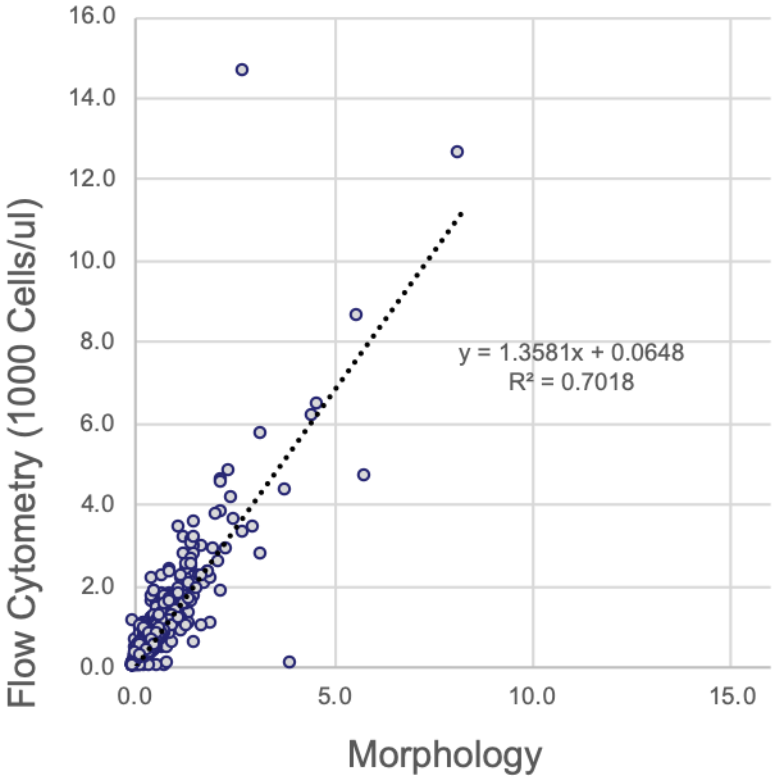
R^2 0.79 – 0.87



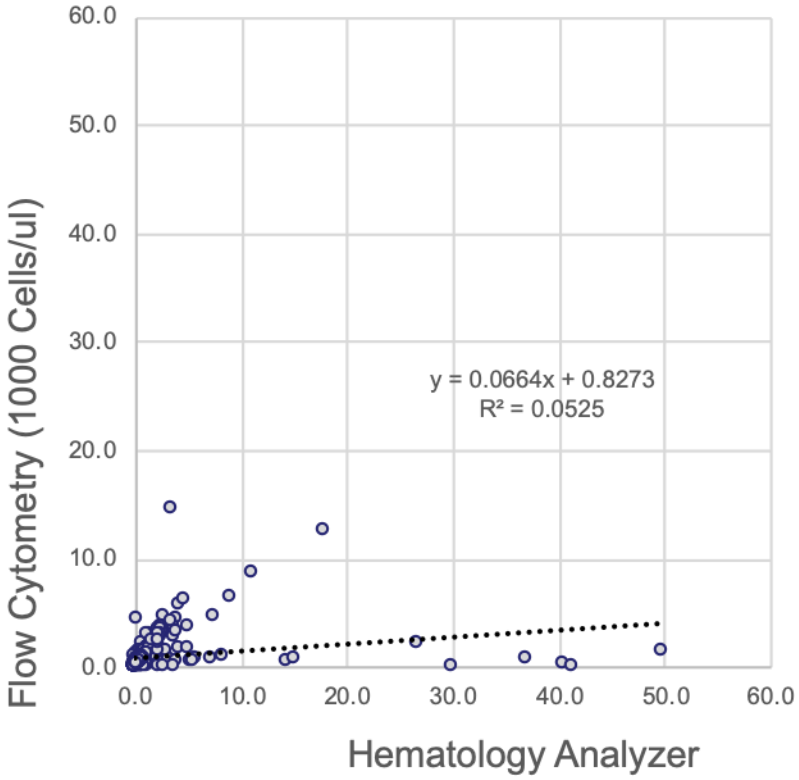


Monocyte Abs

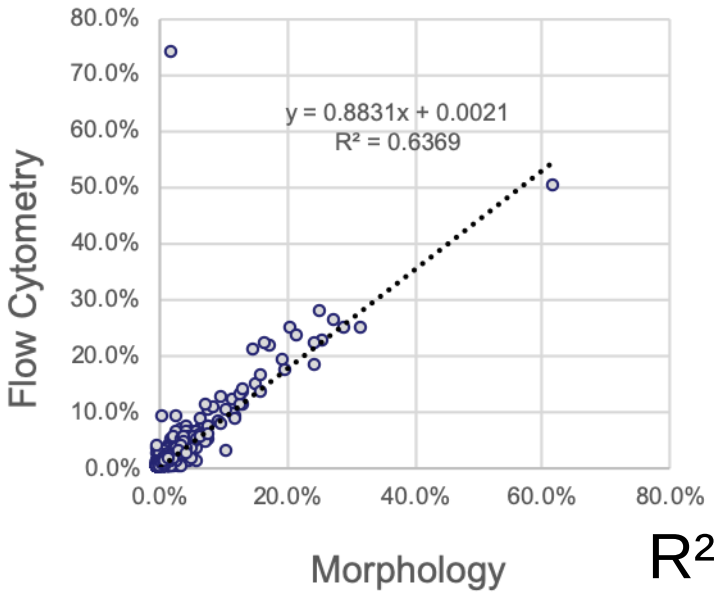
R^2 0.70

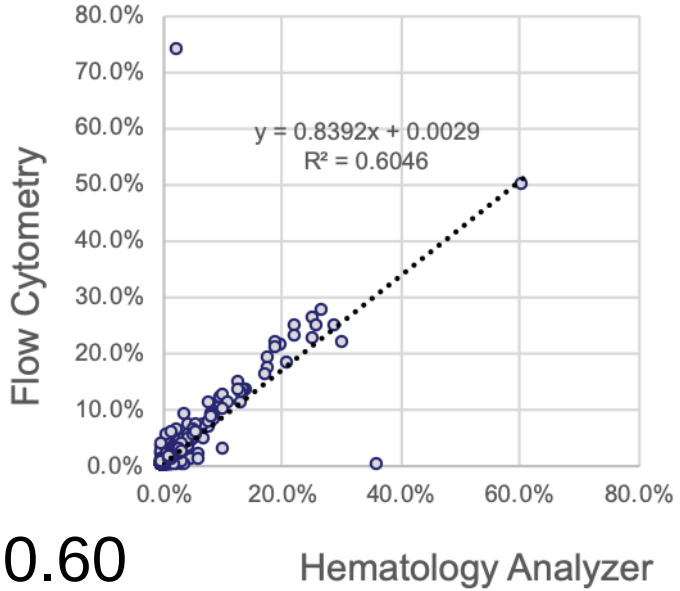


R^2 0.52

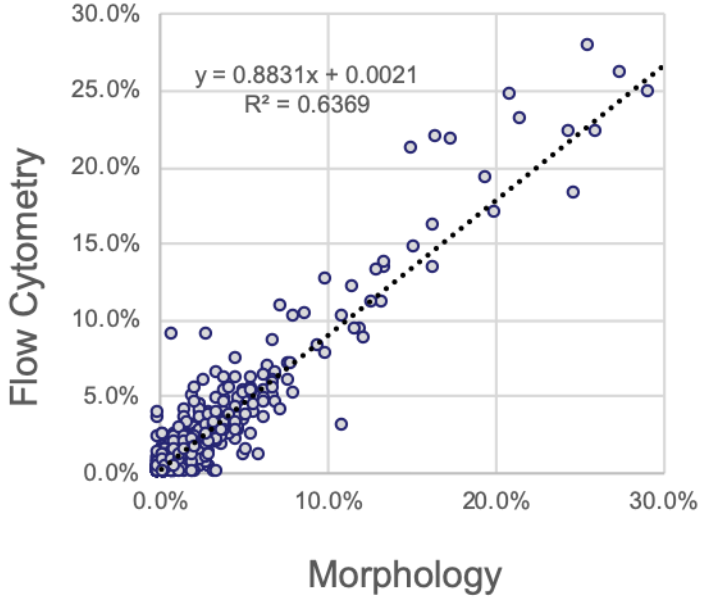


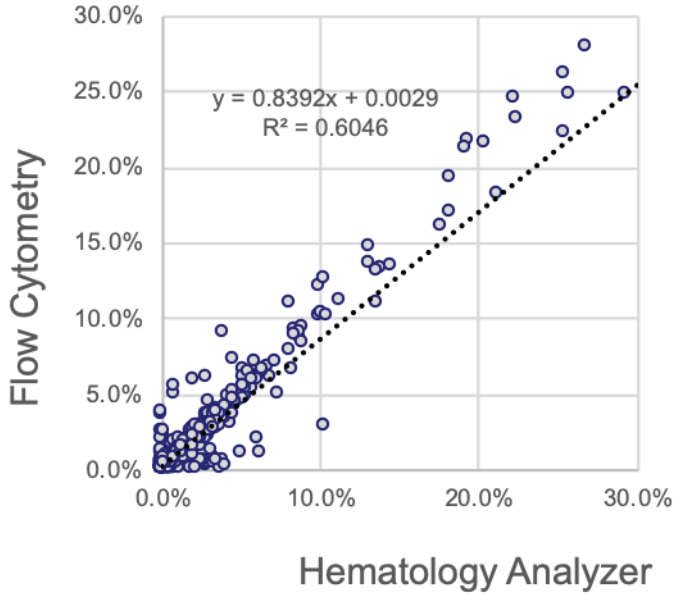
Eosinophils %



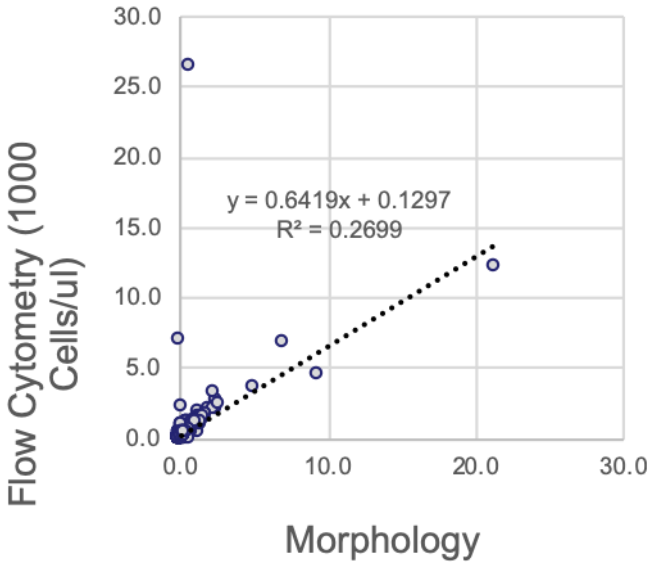


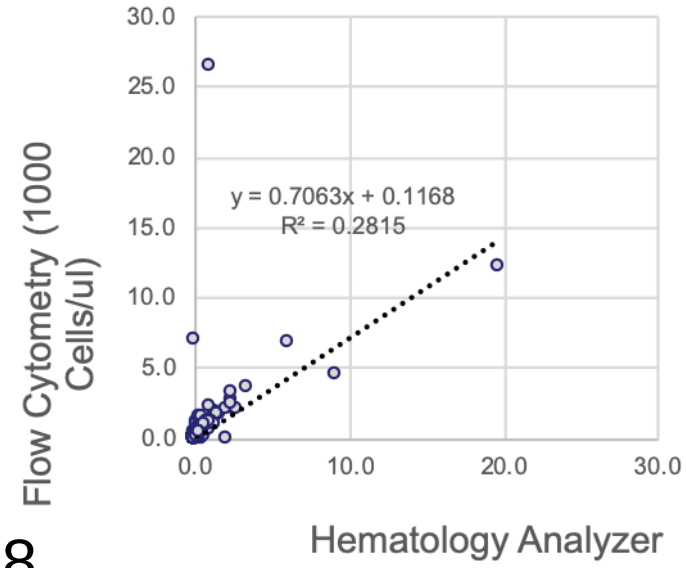
R² 0.64 – 0.60



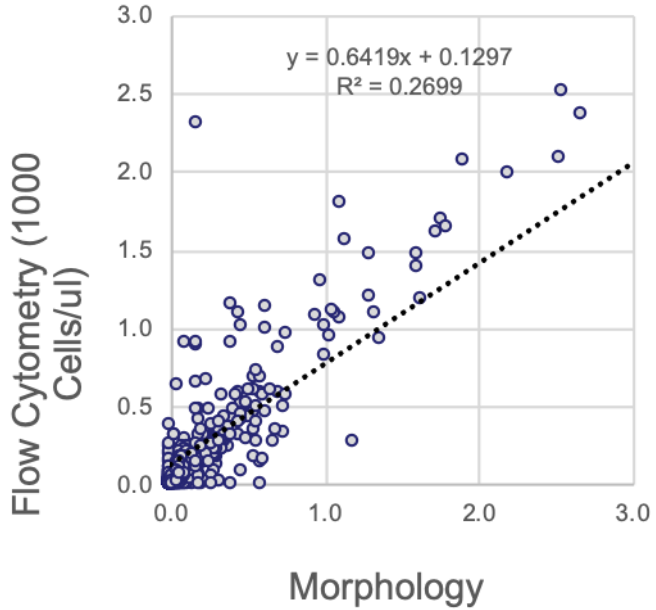


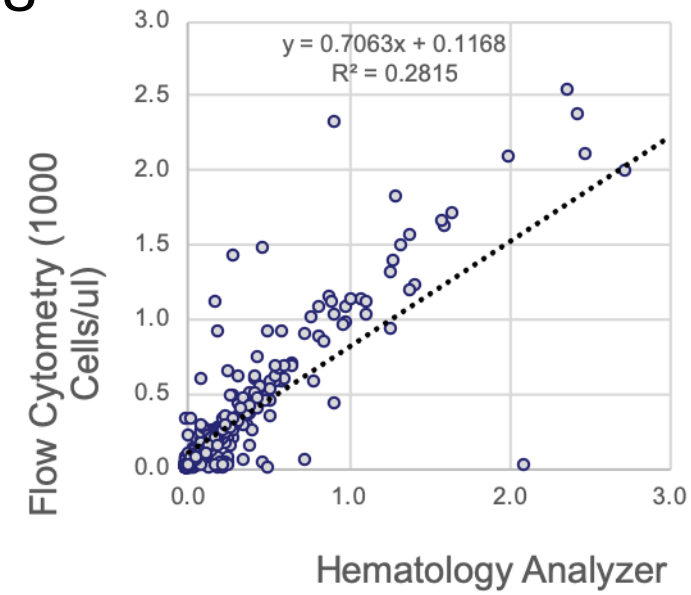
Eosinophils Abs



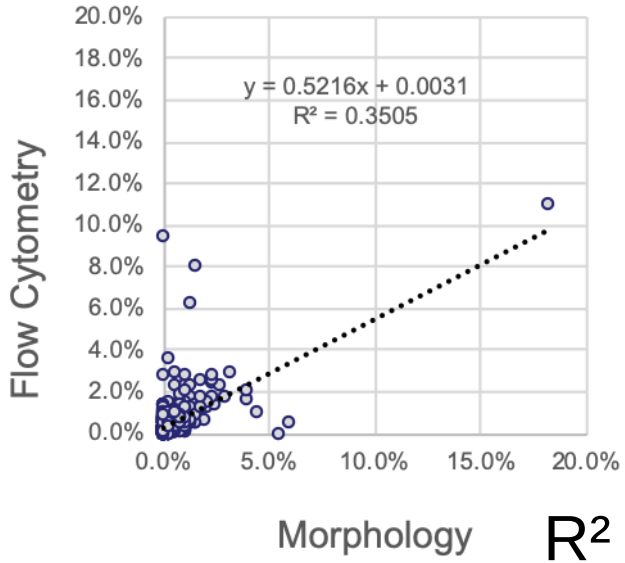


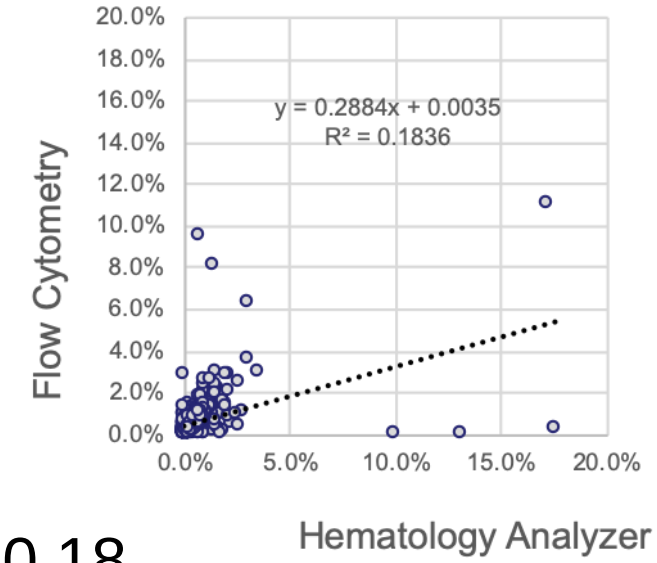
R² 0.28



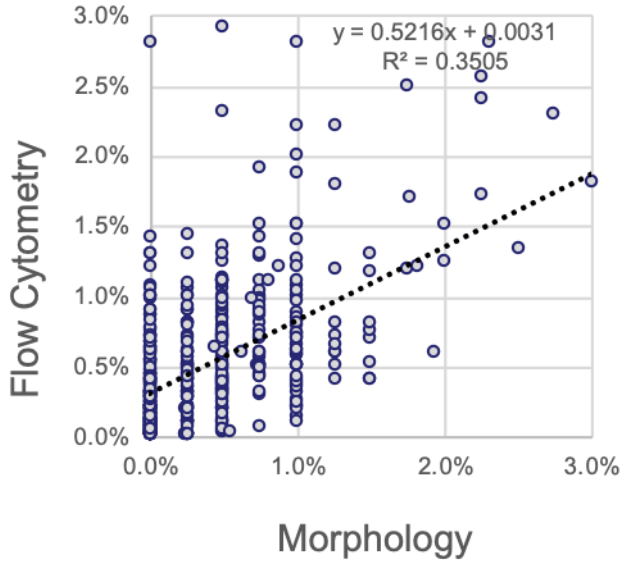


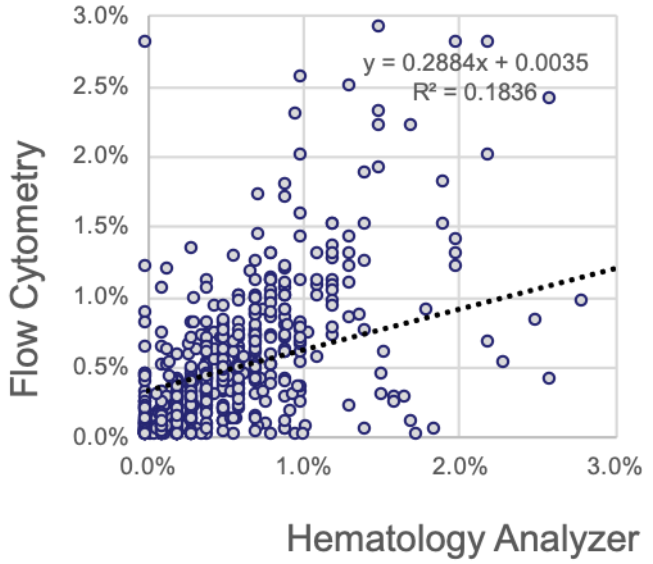
Basophils %



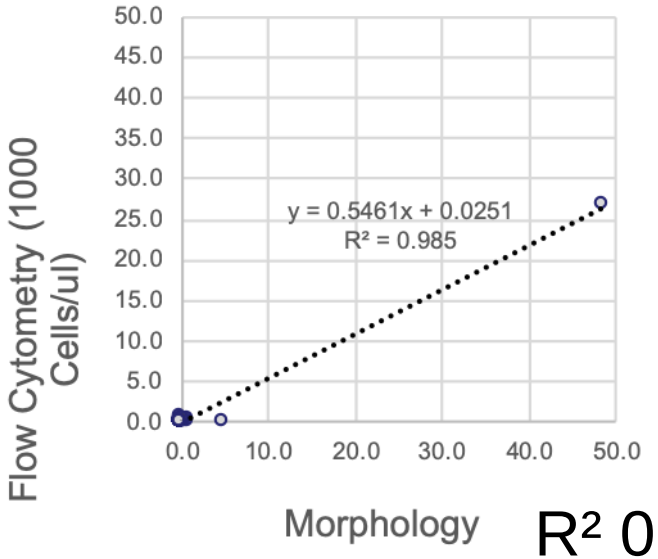


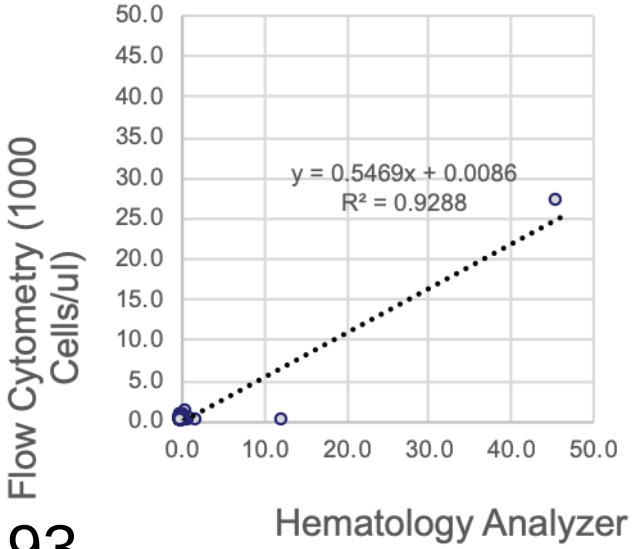
R² 0.35 – 0.18



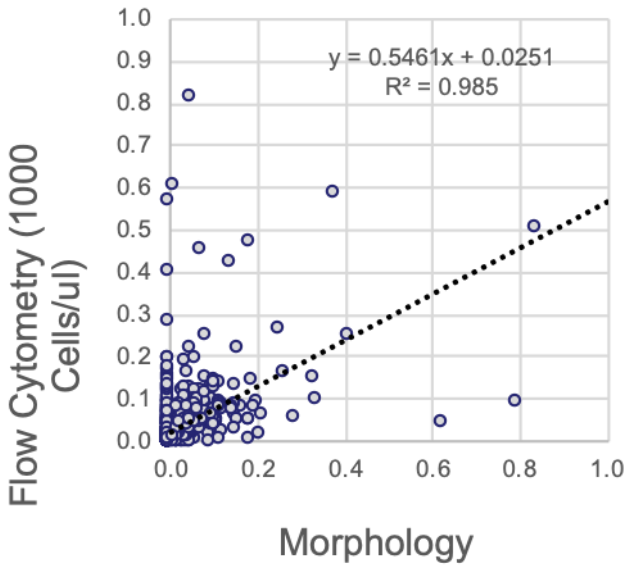


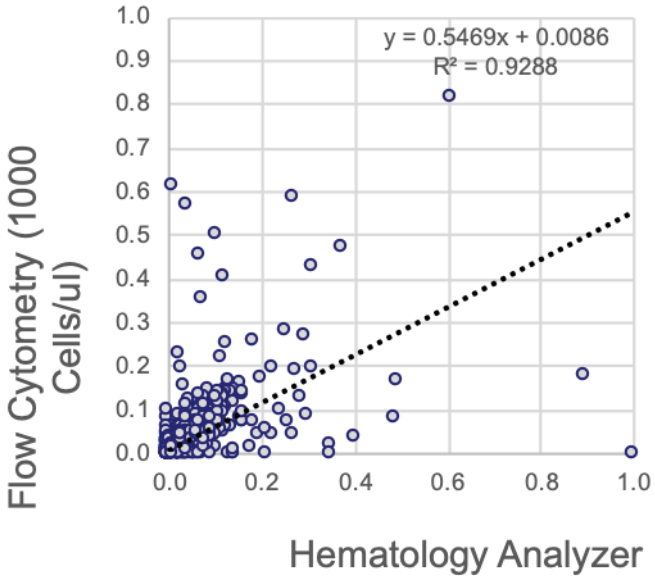
Basophils Abs



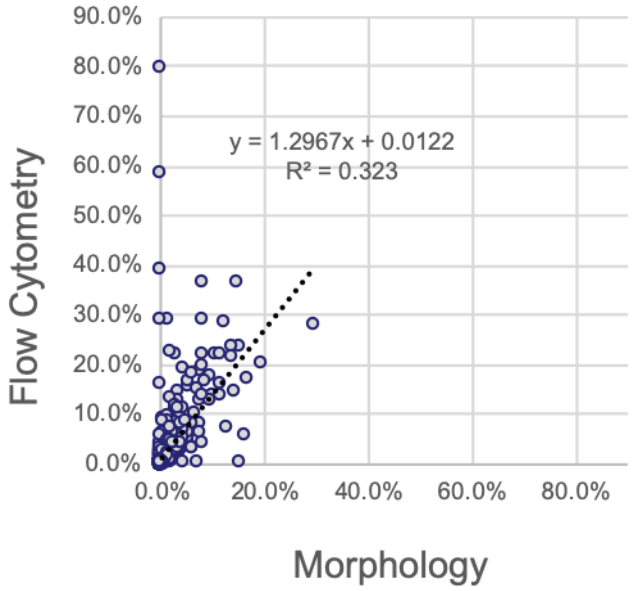


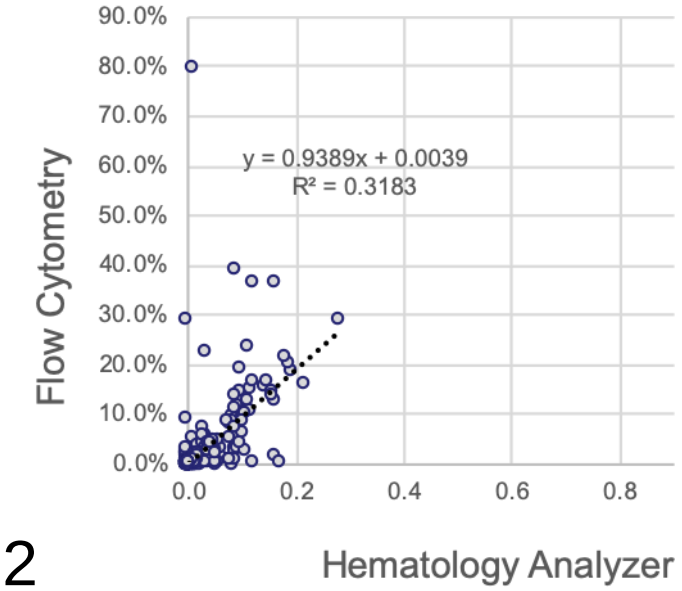
R^2 0.98 – 0.93



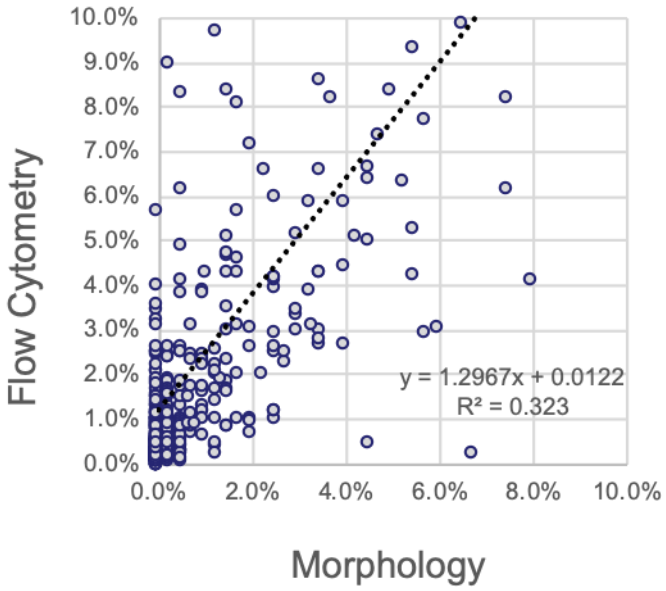


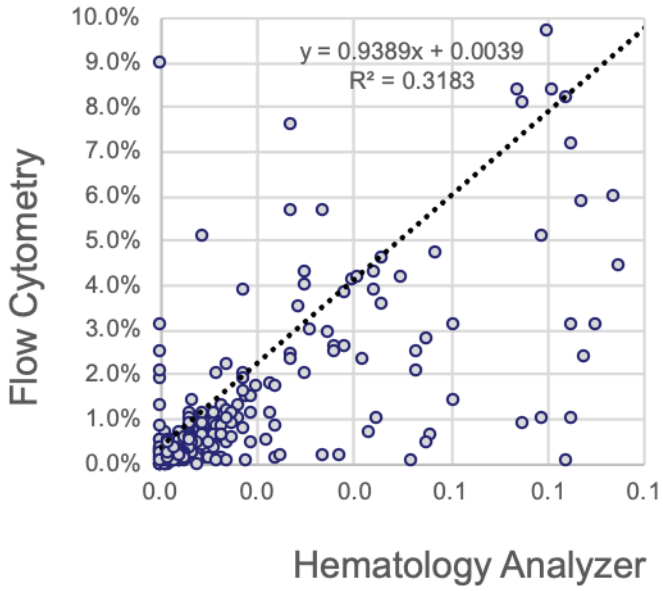
IG %


y = 1.2967x + 0.0122
R² = 0.323
Flow Cytometry
Morphology


y = 0.9389x + 0.0039
R² = 0.3183
Flow Cytometry
Hematology Analyzer

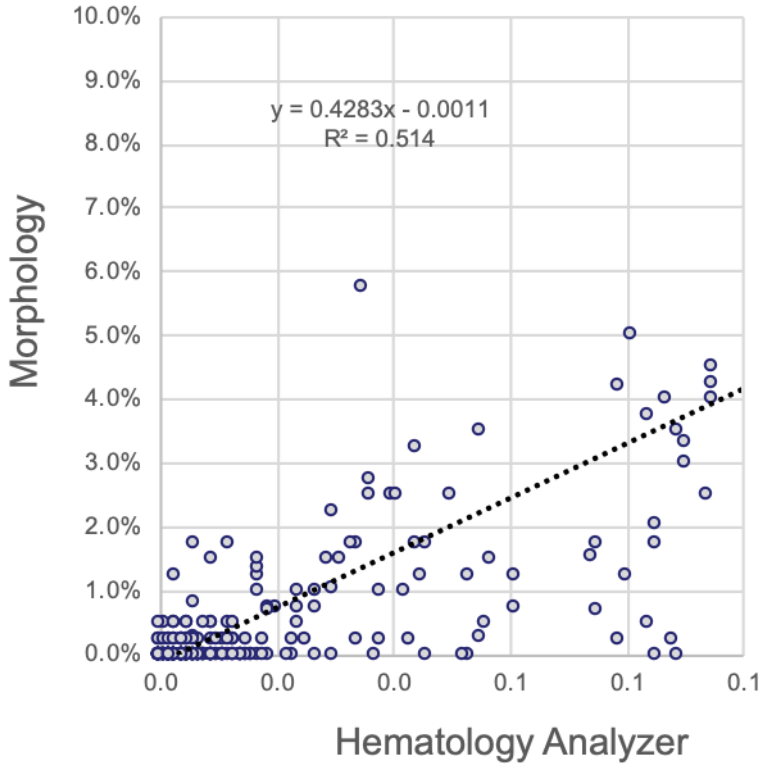
R² 0.32


y = 1.2967x + 0.0122
R² = 0.323
Flow Cytometry
Morphology

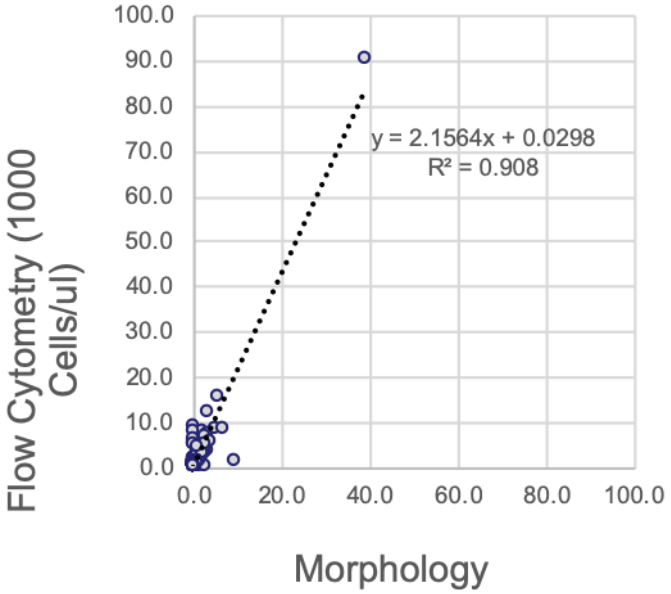

y = 0.9389x + 0.0039
R² = 0.3183
Flow Cytometry
Hematology Analyzer

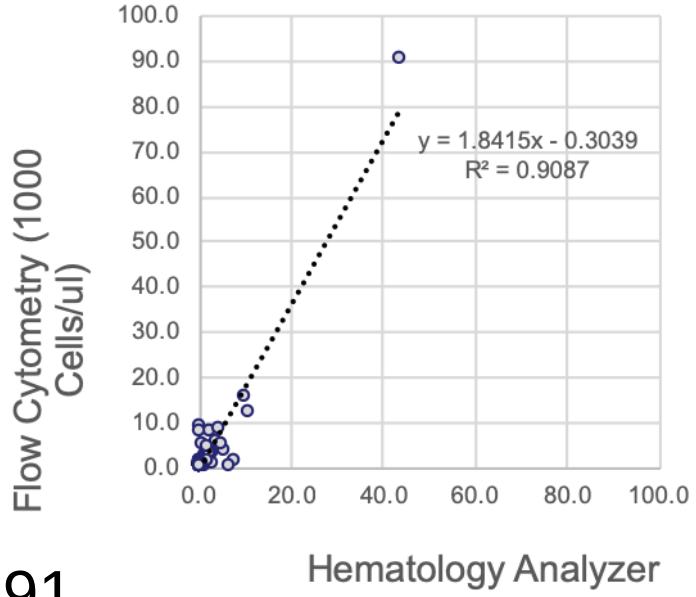
IG %

R^2 0.51

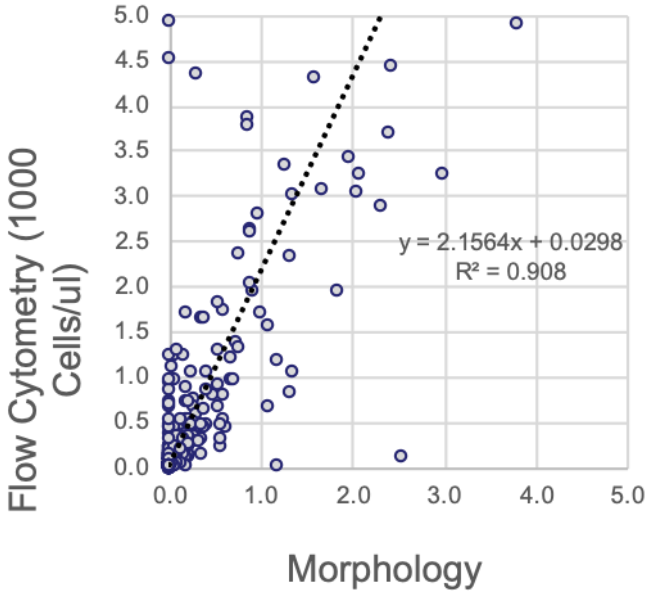

y = 0.4283x - 0.0011
R² = 0.514
Morphology
Hematology Analyzer
10.0%
9.0%
8.0%
7.0%
6.0%
5.0%
4.0%
3.0%
2.0%
1.0%
0.0%
0.0
0.0
0.0
0.1
0.1
0.1

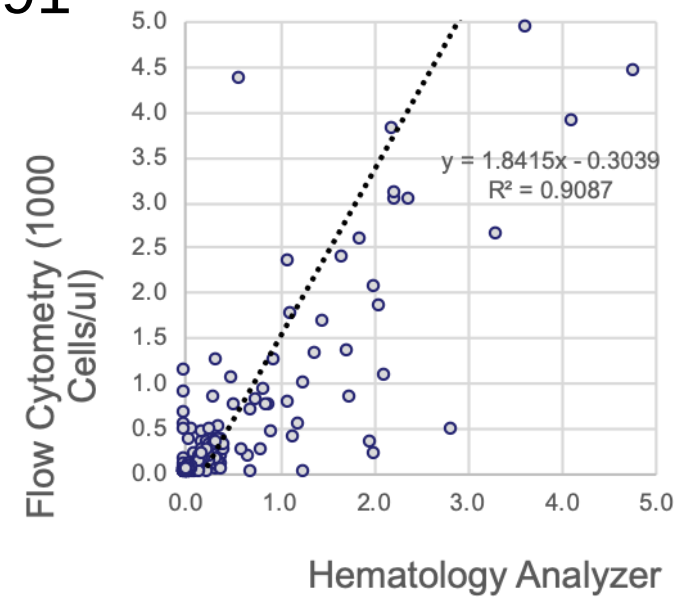
IG Abs


Flow Cytometry (1000 Cells/ul)
Morphology
y = 2.1564x + 0.0298
R² = 0.908
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
20.0
40.0
60.0
80.0
100.0

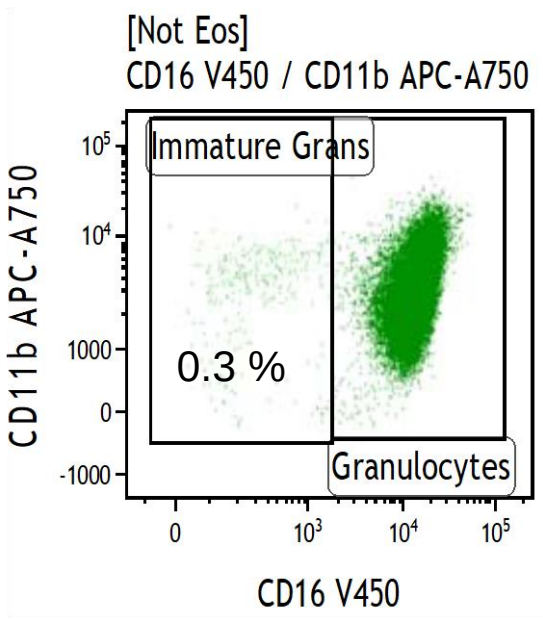

Flow Cytometry (1000 Cells/ul)
Hematology Analyzer
y = 1.8415x - 0.3039
R² = 0.9087
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
20.0
40.0
60.0
80.0
100.0

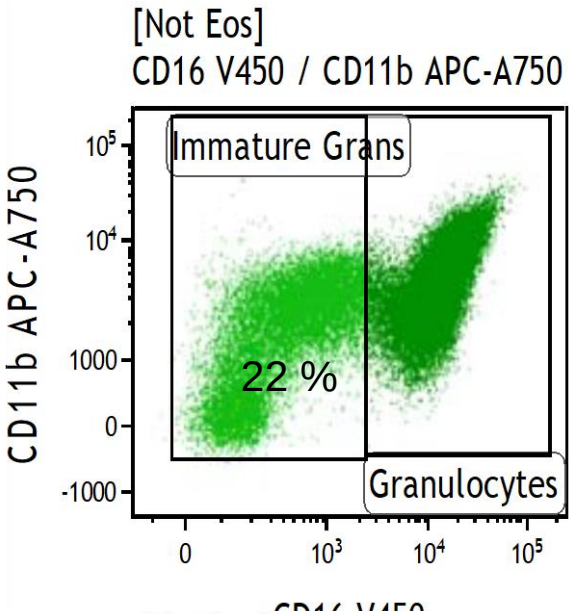
R² 0.91

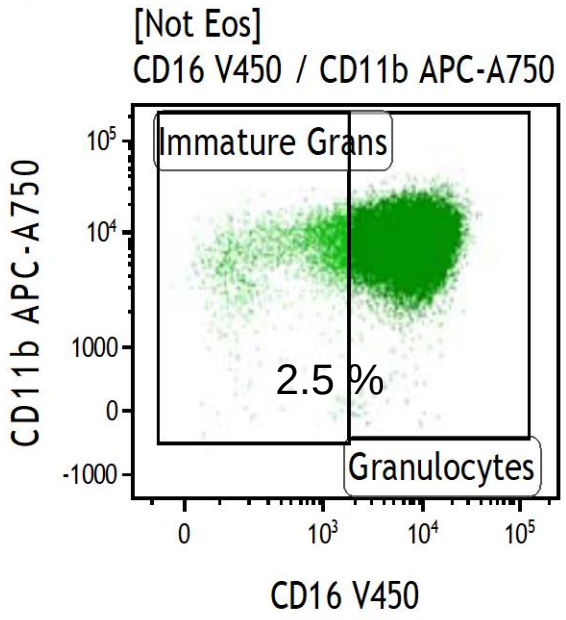

Flow Cytometry (1000 Cells/ul)
Morphology
y = 2.1564x + 0.0298
R² = 0.908
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
1.0
2.0
3.0
4.0
5.0

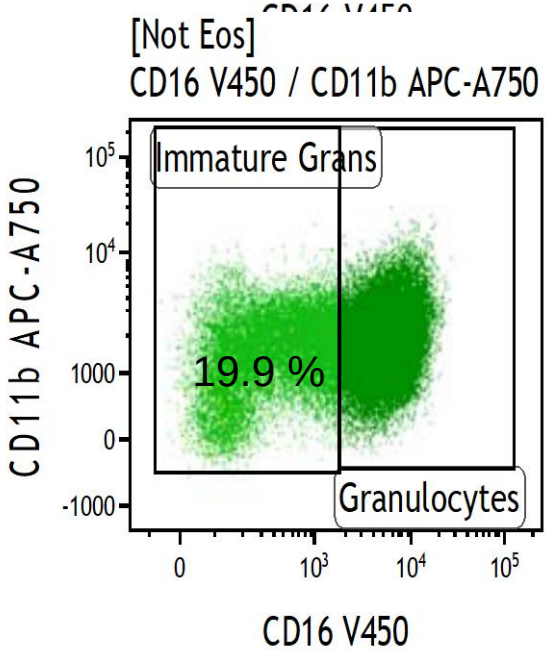

Flow Cytometry (1000 Cells/ul)
Hematology Analyzer
y = 1.8415x - 0.3039
R² = 0.9087
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0.0
1.0
2.0
3.0
4.0
5.0

IG Abs


[Not Eos]
CD16 V450 / CD11b APC-A750
Immature Grans
0.3 %
Granulocytes
CD11b APC-A750
CD16 V450


[Not Eos]
CD16 V450 / CD11b APC-A750
Immature Grans
22 %
Granulocytes
CD11b APC-A750


[Not Eos]
CD16 V450 / CD11b APC-A750
Immature Grans
2.5 %
Granulocytes
CD11b APC-A750
CD16 V450


[Not Eos]
CD16 V450 / CD11b APC-A750
Immature Grans
19.9 %
Granulocytes
CD11b APC-A750
CD16 V450

Accuracy Summary

Population	Morphology	Hematology	Flow Cytometry
WBC count	-	Good	Good
Neutrophils	Good	Good	Good
Lymphocytes	Good	Subset poor	Good
Monocytes	Good	Subset poor	Good
Eosinophils	Fair	Fair	Fair
Basophils	Poor	Poor	Good
IG	Fair	Poor	Fair

Accuracy Summary

Flow reference method at least equivalent to morphology

Discrepancies with analyzers to investigate

Timeline

- Complete Sample Acquisition
  - Missing data from Nantes to complete
- Harmonize Reported Data
  - Raw data from Beijing
  - Complete reporting of all parameters
- Perform Outlier Analysis
  - Identify technical errors
  - Refine gating strategy / cutoff for Eos, Igs, blasts
- Write Draft Manuscript for Review