Flow Cytometry in the Diagnosis of Hematopoietic Neoplasia

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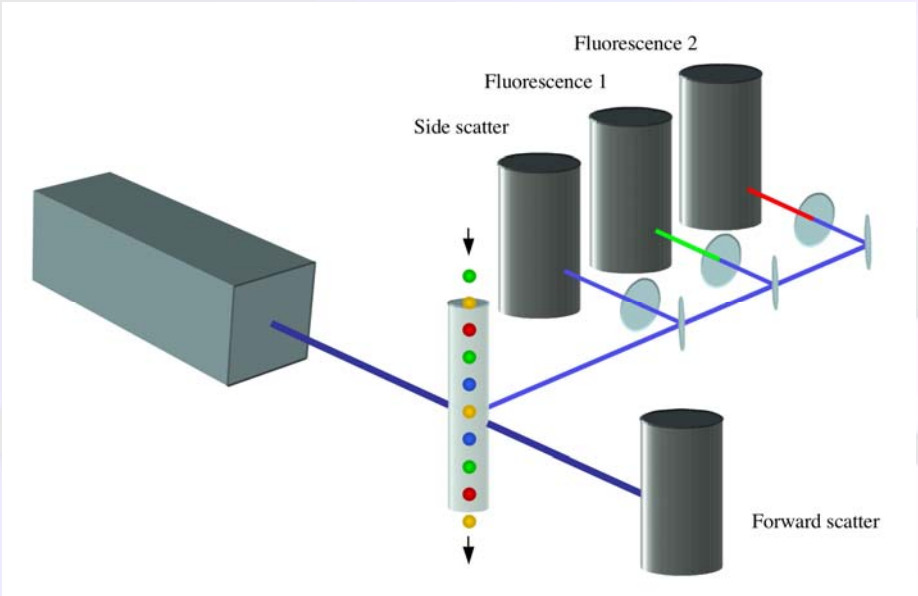
Flow Cytometer



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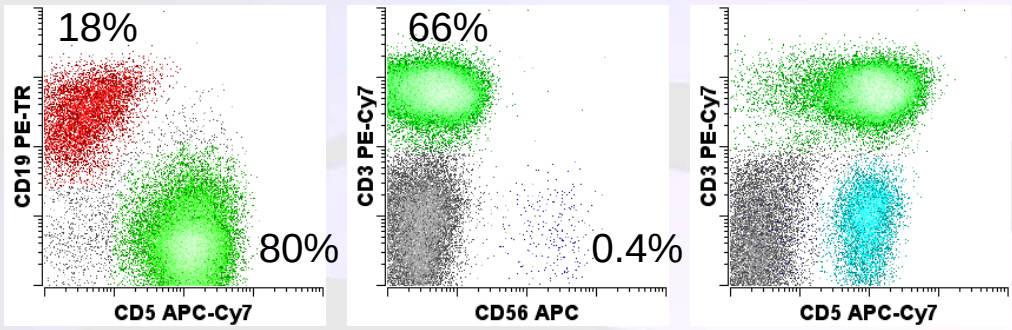
The Power of Flow Cytometry

- Single cell analysis
- Multiparametric
- Rapid
- Quantitative
- Flexible

Clinical Assays

- Lymphocyte subset analysis
- Immunodeficiency
- Stem cell enumeration (CD34)
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Reticulocytes
- Fetal erythrocytes
- HLA crossmatch
- Leukemia and Lymphoma

Inferential reasoning



- Insensitive
- Misattribution if assumptions incorrect

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Direct Observation

- Combination of reagents uniquely identifies cell type, lineage and maturational stage
  - Emphasize normal maturational patterns
- Direct determination of immunophenotype without inference
- Improvement in sensitivity and specificity
- More simultaneous fluorochromes improves

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Multiparametric Flow Cytometry

- More accurate population identification
  - Greater informational content
- Make better use of small specimens
  - Fewer cells, more information
- Process fewer tubes
  - Save on reagents, tech and instrument time
- Collect large number of events efficiently
- Allow standardized reagent combinations

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How Many Colors are Enough?

- Ideal
  - Add all reagents of interest into single tube
- Real
  - Too many hematopoietic disorders
  - Single comprehensive tube per cell lineage
  - Focus on screening tubes

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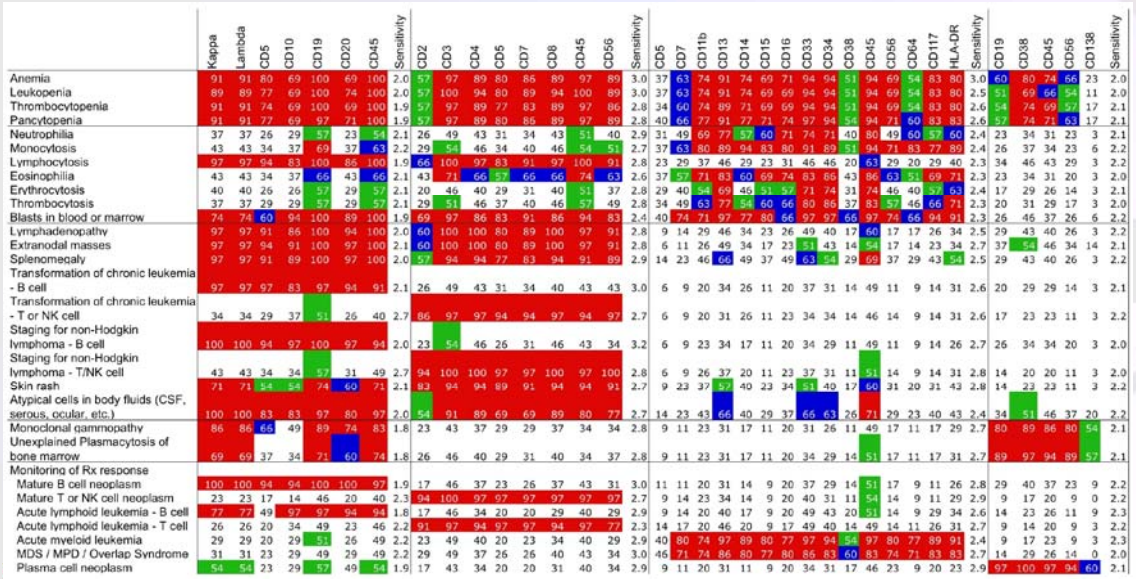
Bethesda International Consensus Conference



	Kappa	Lambda	CD5	CD10	CD19	CD20	CD45	Sensitivity	CD2	CD3	CD4	CD5	CD7	CD8	CD45	CD56	Sensitivity	CD5	CD7	CD11b	CD13	CD14	CD15	CD16	CD33	CD34	CD38	CD45	CD56	CD64	CD117	HLA-DR	Sensitivity	CD19	CD38	CD45	CD56	CD138	Sensitivity
Anemia	91	91	80	69	100	69	100	2.0	57	97	89	80	86	89	97	89	3.0	37	63	74	91	74	69	71	94	94	51	94	69	54	83	80	3.0	60	80	74	66	23	2.0
Leukopenia	89	89	77	69	100	74	100	2.0	57	100	94	80	89	94	100	89	3.0	37	63	74	91	74	69	69	94	94	51	94	69	54	83	80	2.5	51	69	66	54	11	2.0
Thrombocytopenia	91	91	74	69	100	69	100	1.9	57	97	89	77	83	89	97	86	2.8	34	60	74	89	71	69	69	94	94	51	94	69	54	83	80	2.6	54	74	69	57	17	2.1
Pancytopenia	91	91	77	69	97	71	100	1.9	57	97	89	80	86	89	97	89	2.8	40	66	77	91	77	71	74	97	94	54	94	71	60	83	83	2.6	57	74	71	63	17	2.1
Neutrophilia	37	37	26	29	57	23	54	2.1	26	49	43	31	34	43	51	40	2.9	31	49	69	77	57	60	71	74	71	40	80	49	60	57	60	2.4	23	34	31	23	3	2.1
Monocytosis	43	43	34	37	69	37	63	2.2	29	54	46	34	40	46	54	51	2.7	37	63	80	89	94	83	80	91	89	51	94	71	83	77	89	2.4	26	37	34	23	6	2.2
Lymphocytosis	97	97	94	83	100	86	100	1.9	66	100	97	83	91	97	100	91	2.8	23	29	37	46	29	23	31	46	46	20	63	29	20	29	40	2.3	34	46	43	29	3	2.2
Eosinophilia	43	43	34	37	66	43	66	2.1	43	71	66	57	66	66	74	63	2.6	37	57	71	83	60	69	74	83	86	43	86	63	51	69	71	2.3	23	34	31	20	3	2.0
Erythrocytosis	40	40	26	26	57	29	57	2.1	20	46	40	29	31	40	51	37	2.8	29	40	54	69	46	51	57	71	74	31	74	46	40	57	63	2.4	17	29	26	14	3	2.1
Thrombocytosis	37	37	29	29	57	29	57	2.1	29	51	46	37	40	46	57	49	2.8	34	49	63	77	54	60	66	80	86	37	83	57	46	66	71	2.3	20	31	29	17	3	2.0
Blasts in blood or marrow	74	74	60	94	100	89	100	1.9	69	97	86	83	91	86	94	83	2.4	40	74	71	97	77	80	66	97	97	66	97	74	66	94	91	2.3	26	46	37	26	6	2.2
Lymphadenopathy	97	97	91	86	100	94	100	2.0	60	100	100	80	89	100	97	91	2.8	9	14	29	46	34	23	26	49	40	17	60	17	17	26	34	2.5	29	43	40	26	3	2.2
Extranodal masses	97	97	94	91	100	97	100	2.1	60	100	100	80	89	100	97	91	2.8	6	11	26	49	34	17	23	51	43	14	54	17	14	23	34	2.7	37	54	46	34	14	2.1
Splenomegaly	97	97	91	89	100	97	100	2.0	57	94	94	77	83	94	91	89	2.9	14	23	46	66	49	37	49	63	54	29	69	37	29	43	54	2.5	29	43	40	26	3	2.2
Transformation of chronic leukemia - B cell	97	97	97	83	97	94	91	2.1	26	49	43	31	34	40	43	43	3.0	6	9	20	34	26	11	20	37	31	14	49	11	9	14	31	2.6	20	29	29	14	3	2.1
Transformation of chronic leukemia - T or NK cell	34	34	29	37	51	26	40	2.7	86	97	97	94	94	97	94	97	2.7	6	9	20	31	26	11	23	34	34	14	46	14	9	14	31	2.6	17	23	23	11	3	2.2
Staging for non-Hodgkin lymphoma - B cell	100	100	94	97	100	97	94	2.0	23	54	46	26	31	46	43	34	3.2	6	9	23	34	17	11	20	34	29	11	49	11	9	14	26	2.7	26	34	34	20	3	2.0
Staging for non-Hodgkin lymphoma - T/NK cell	43	43	34	34	57	31	49	2.7	94	100	100	97	97	100	97	100	2.8	6	9	26	37	20	11	23	37	31	11	51	14	9	14	31	2.8	14	20	20	11	3	2.0
Skin rash	71	71	54	54	74	60	71	2.1	83	94	94	89	91	94	94	91	2.7	9	23	37	57	40	23	34	51	49	17	60	31	20	31	43	2.8	14	23	23	11	3	2.2
Atypical cells in body fluids (CSF, serous, ocular, etc.)	100	100	83	83	97	80	97	2.0	54	91	89	69	69	89	80	77	2.7	14	23	43	66	40	29	37	66	63	26	71	29	23	40	43	2.4	34	51	46	37	20	2.2
Monoclonal gammopathy	86	86	66	49	89	74	83	1.8	23	43	37	29	29	37	34	34	2.8	9	11	23	31	17	11	20	31	26	11	49	17	11	17	29	2.7	80	89	86	80	54	2.1
Unexplained Plasmacytosis of bone marrow	69	69	37	34	71	60	74	1.8	26	46	40	29	31	40	34	37	2.8	9	11	23	31	17	11	20	34	29	14	51	17	11	17	31	2.7	89	97	94	89	57	2.1
Monitoring of Rx response																																							
Mature B cell neoplasm	100	100	94	94	100	100	97	1.9	17	46	37	23	26	37	43	31	3.0	11	11	20	31	14	9	20	37	29	14	51	17	9	11	26	2.8	29	40	37	23	9	2.2
Mature T or NK cell neoplasm	23	23	17	14	46	20	40	2.3	94	100	97	97	97	97	97	97	2.7	9	14	23	34	14	9	20	40	31	11	54	14	9	11	29	2.9	9	17	20	9	0	2.2
Acute lymphoid leukemia - B cell	77	77	49	97	97	94	94	1.8	17	46	34	20	20	29	40	29	2.9	9	14	20	40	17	9	20	49	43	20	51	14	9	29	34	2.6	14	23	26	11	9	2.3
Acute lymphoid leukemia - T cell	26	26	20	34	49	23	46	2.2	91	97	94	97	97	94	97	77	2.3	14	17	20	46	20	9	17	49	40	14	49	14	11	26	31	2.7	9	14	20	9	3	2.2
Acute myeloid leukemia	29	29	20	29	54	26	49	2.2	23	49	40	20	23	34	40	29	2.9	40	80	74	97	89	80	77	97	94	54	97	80	77	89	91	2.4	9	17	23	9	3	2.3
MDS / MPD / Overlap Syndrome	31	31	23	29	49	29	49	2.2	29	49	37	26	26	40	43	34	3.0	46	71	74	86	80	77	80	86	83	60	83	74	71	83	83	2.7	14	29	26	14	0	2.0
Plasma cell neoplasm	54	54	23	29	57	49	54	1.9	17	43	34	20	20	31	40	34	2.9	9	11	20	31	11	9	20	34	31	17	46	23	9	20	23	2.9	97	100	97	94	60	2.1

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N = 35

Euroflow

TABLE 1. Single tube EuroFlow screening combinations for acute leukemias (ALOT)*

Pacific Blue	Pacific Orange	FITC	PE	PerCP-Cy5.5	PE-Cy7	APC	APC-H7
cyCD3	**CD45**	cyMPO	cyCD79a	**CD34**	**CD19**	CD7	**smCD3**

TABLE 7. Multi-tube EuroFlow classification combinations for AML/MDS. *

Tube	Pacific Blue	Pacific Orange	FITC	PE	PerC-Cy5.5	PE-Cy7	APC	APC-H7	Aim**
AML/MDS									
1	**HLADR**	**CD45**	CD16	CD13	**CD34**	**CD117**	CD11b	CD10	Diagnosis and subclassification of AML and PNH especially focused on neutrophilic lineage
2	**HLADR**	**CD45**	CD35	CD64	**CD34**	**CD117**	IREM2	CD14	Diagnosis and subclassification of AML and PNH especially focussed on monocytic lineage
3	**HLADR**	**CD45**	CD36	CD105	**CD34**	**CD117**	CD33	CD71	Diagnosis and subclassification of AML especially focused on erythroid lineage
4	**HLADR**	**CD45**	nuTdT	CD56	**CD34**	**CD117**	CD7	CD19	Aberrant expression of lymphoid-associated markers and abnormal lymphoid maturation
AML									
5	**HLADR**	**CD45**	CD15	NG2	**CD34**	**CD117**	CD22	CD38	Aberrant expression of markers; detection of stem cells
6	**HLADR**	**CD45**	CD42a and CD61	CD203c	**CD34**	**CD117**	CD123	CD4	Diagnosis and subclassification of AML especially focused on megakaryocytic, basophilic, and plasmacytoid dendritic lineages
AML-M7									
7	**HLADR**	**CD45**	CD41	CD25	**CD34**	**CD117**	CD42b	CD9	Characterization of AML-M7, mastocytosis

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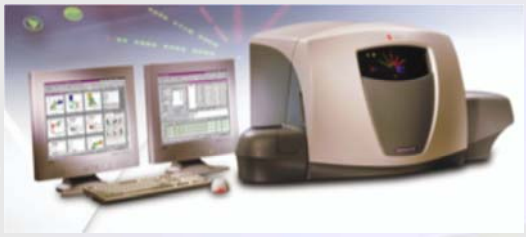
Instrumentation



Beckman-Coulter FC500
5 colors - 1 or 2 lasers

Becton-Dickinson FACSCanto
I - 6 colors, 2 lasers
II - 8 colors, 3 lasers

Beckman-Coulter Gallios
10 colors - 3 lasers

Becton-Dickinson LSRII
~20 color, up to 7 lasers

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Panel Design

	PB	FITC	PE	PE-TR	PE55	PE7	A594	APC	A700	APC7
B cells	45	κ	λ	19	34	20	38	10	-	5
T cells	45	2	7	34	8	3	4	56	-	5
Blasts	DR	15	33	19	117	13	38	34	71	45
Myeloid	DR	64	123	4	14	13	38	34	16	45

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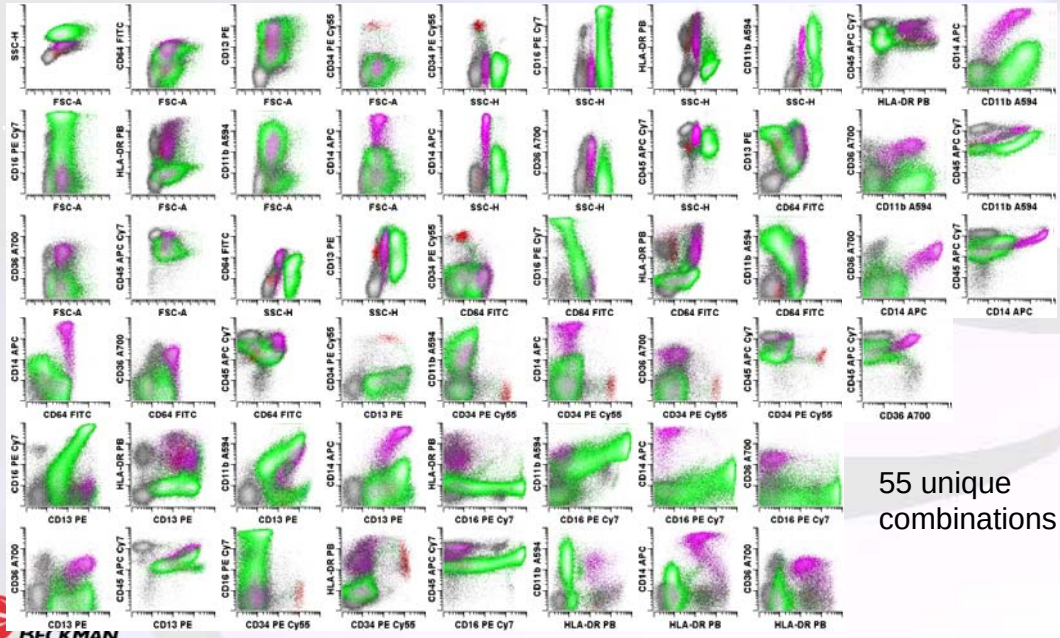
9 Color Flow Cytometry



55 unique combinations

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Abnormal population identification

- Normal
  - Antigens expressed in consistent and reproducible patterns with maturation
- Neoplastic
  - Increased or decreased normal antigens
  - Asynchronous maturational expression
  - Aberrant antigen expression
  - Homogeneous expression

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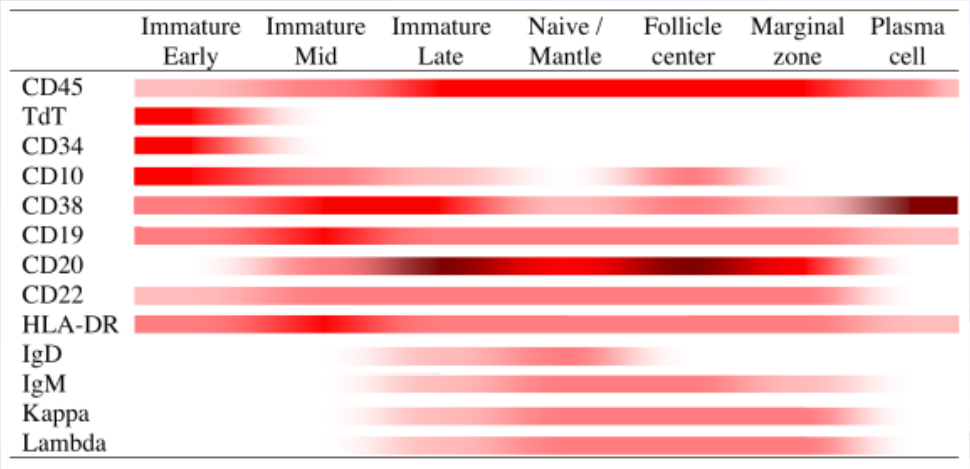
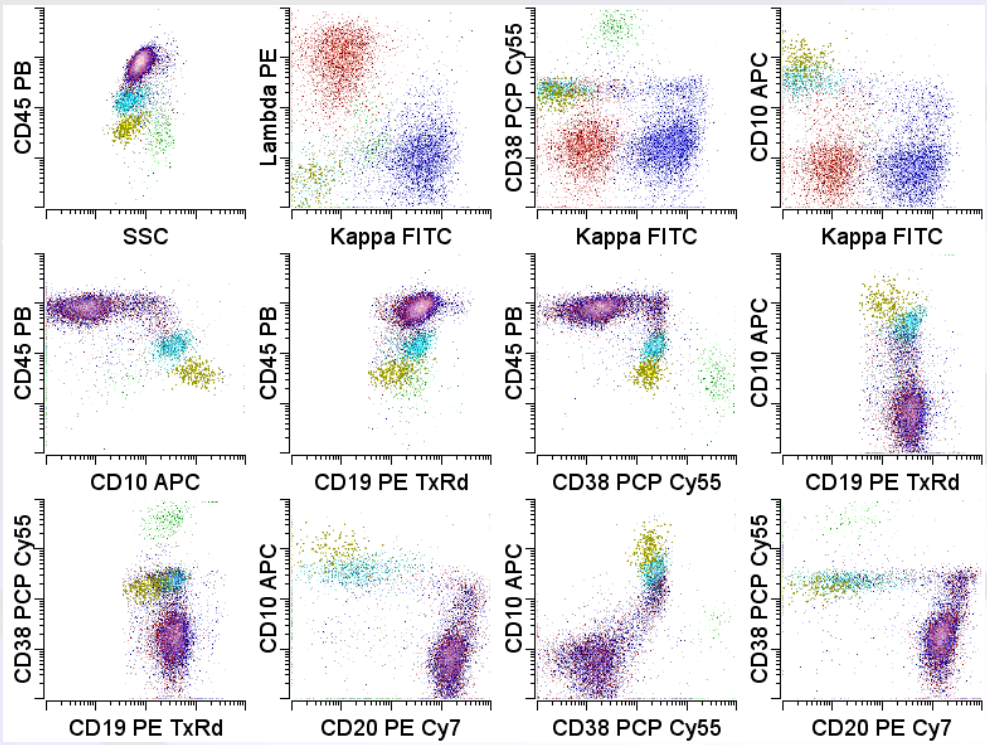
Normal B cell Maturation

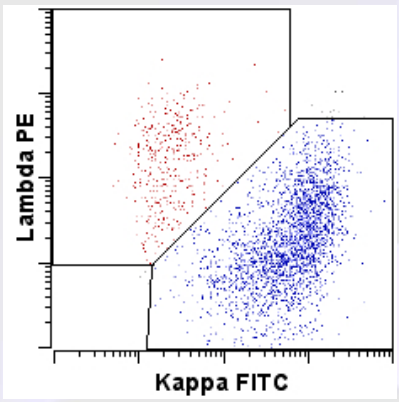


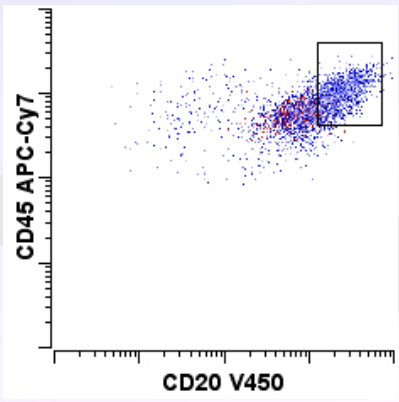
Wood and Borowitz (2006) Henry's Laboratory Medicine



Wood, Methods in Cell Biology, 2004

B cell clonality





10-10531

- Color kappa and lambda
- Look for discrete abnormal B cell populations
- Identify antigenic aberrancy in the context of clonality
- Demonstration of clonality is not necessary

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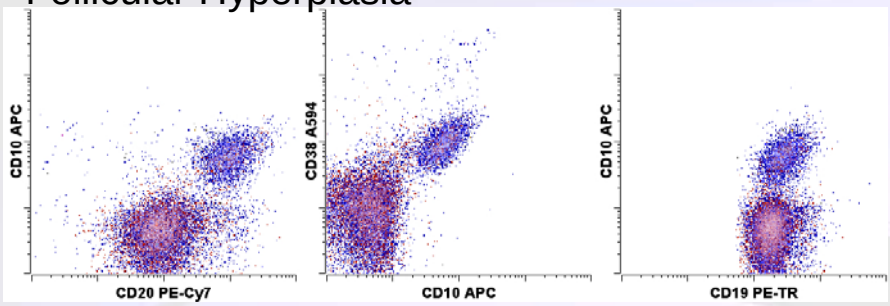
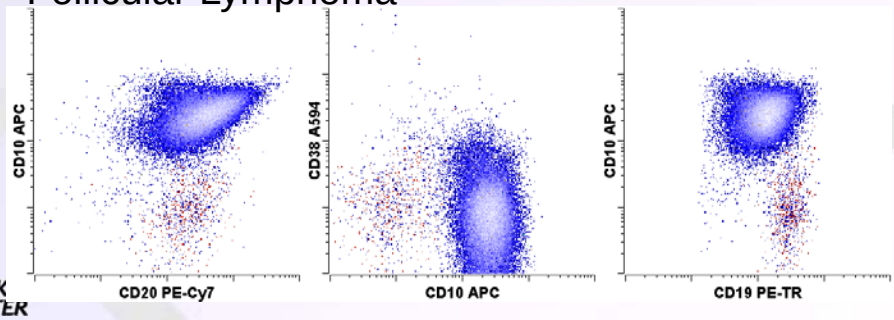
Follicle Center B cells

Follicular Hyperplasia



08-03324

Follicular Lymphoma



08-01359

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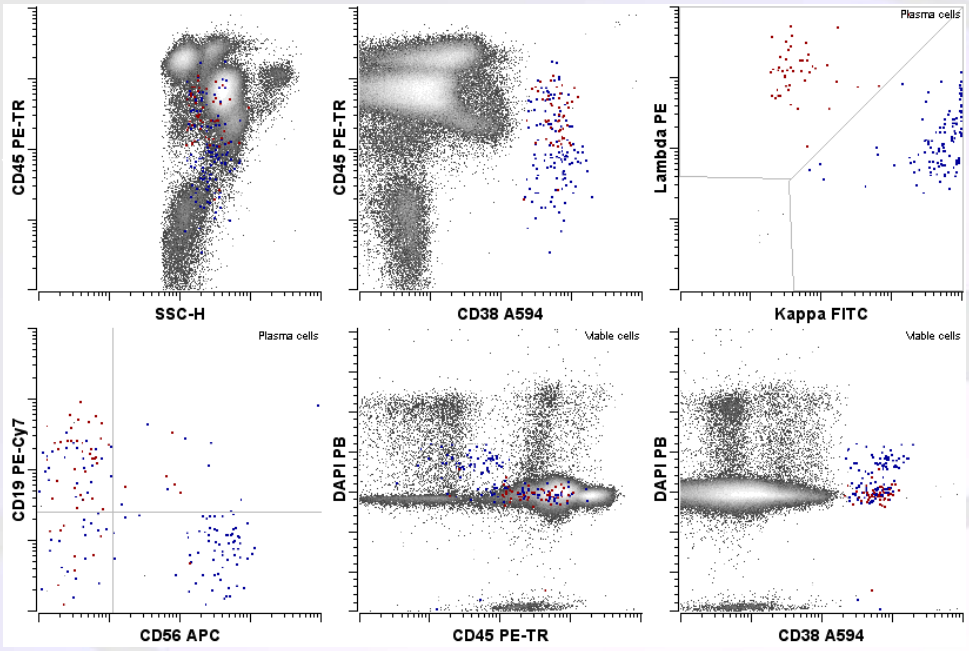
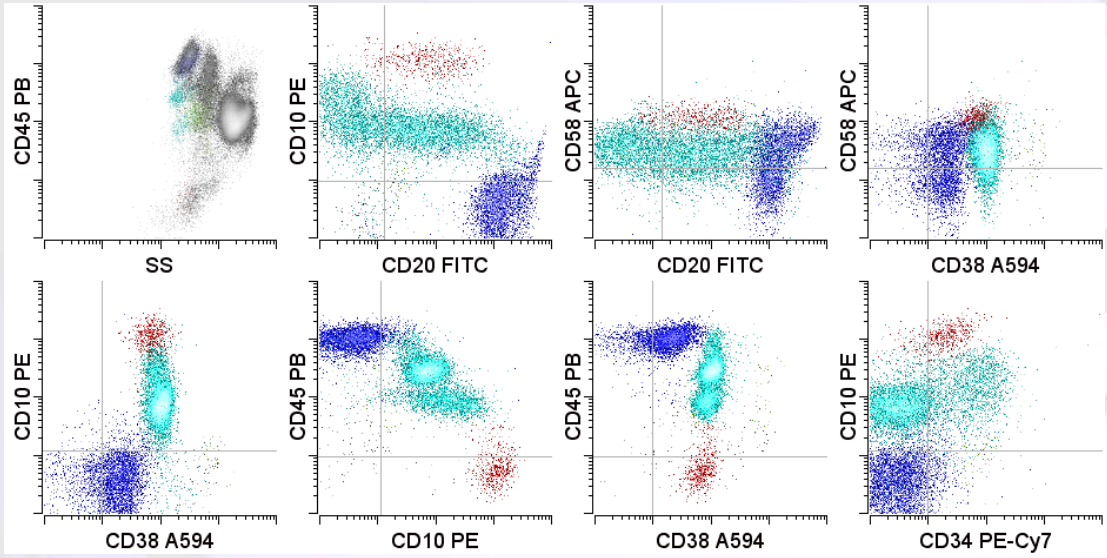
Plasma Cell Neoplasm



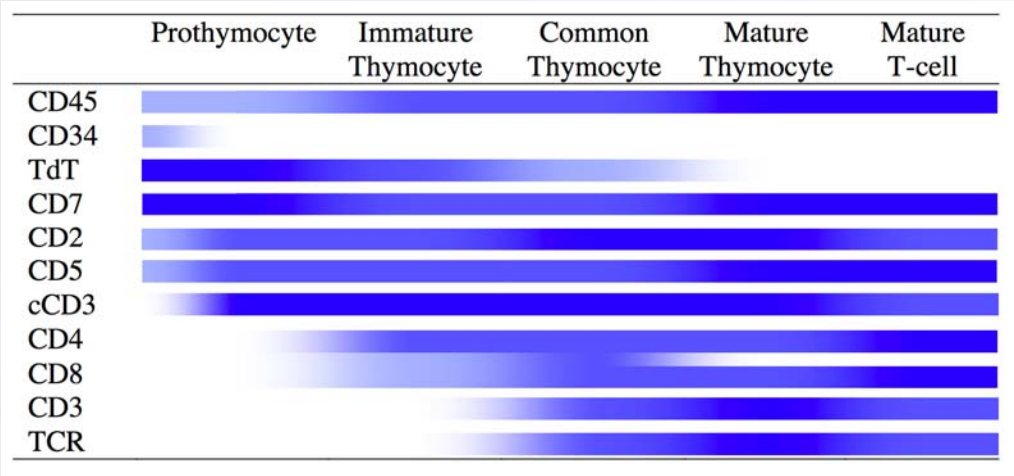
0.05% abnormal plasma cells

ALL MRD



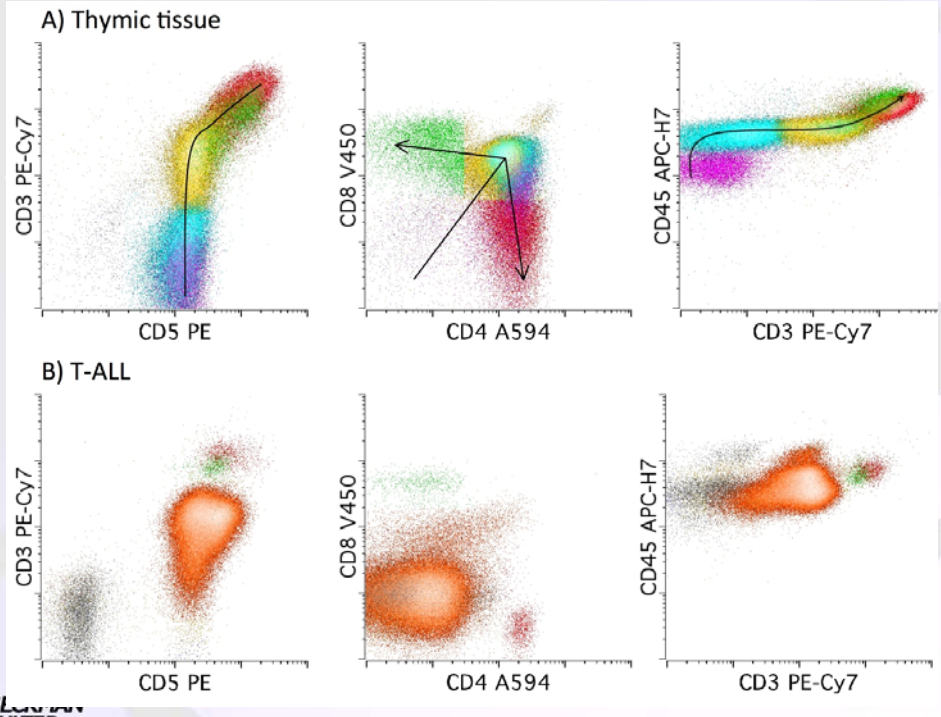
0.1% abnormal immature B cells

Normal T cell Maturation



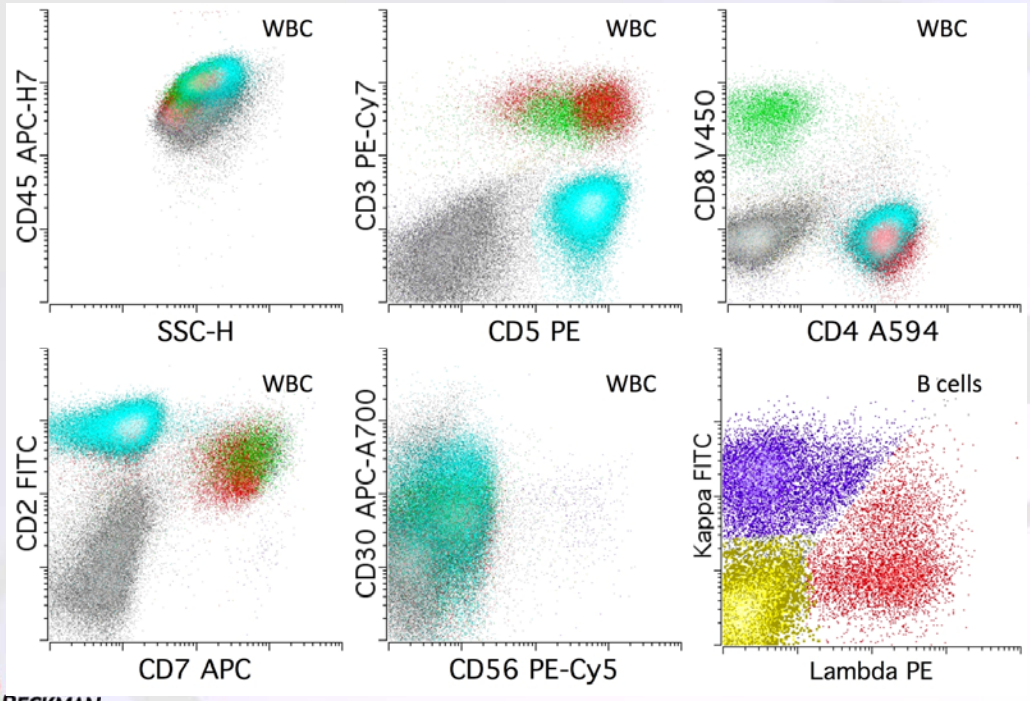
Wood and Borowitz (2010) Henry's Laboratory Medicine

Precursor T-cell Lymphoblastic Leukemia



Cherian and Wood (2012) Flow Cytometry in Evaluation of Hematopoietic Neoplasms: A Case-Based Approach

Angioimmunoblastic T-cell Lymphoma

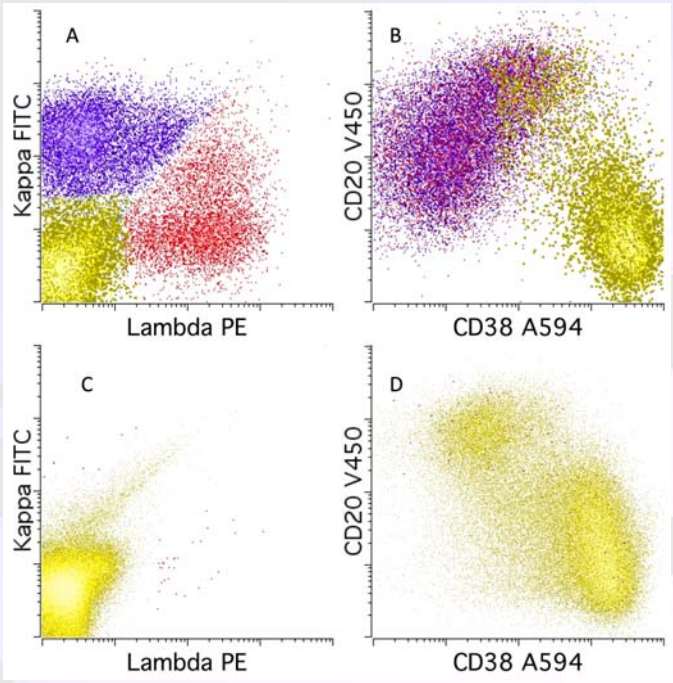


Cherian and Wood (2012) Flow Cytometry in Evaluation of Hematopoietic Neoplasms: A Case-Based Approach

Angioimmunoblastic T-cell Lymphoma

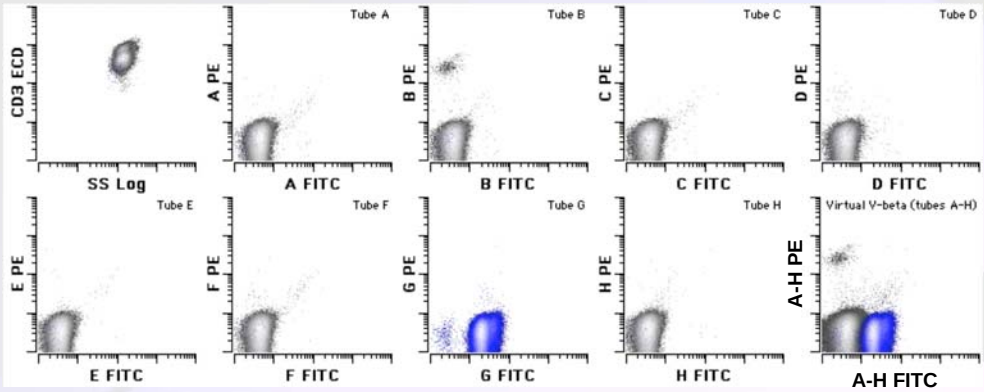
Diagnosis

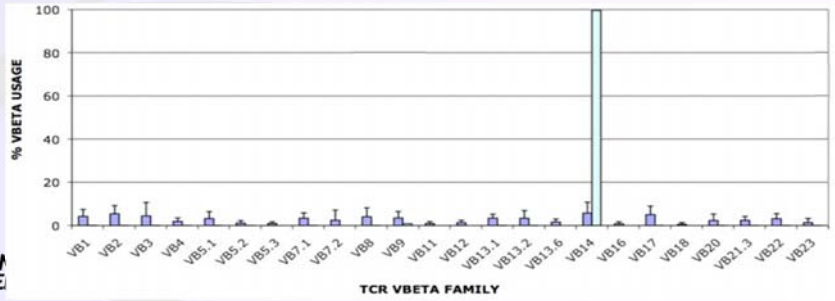
Relapse



Cherian and Wood (2012) Flow Cytometry in Evaluation of Hematopoietic Neoplasms: A Case-Based Approach

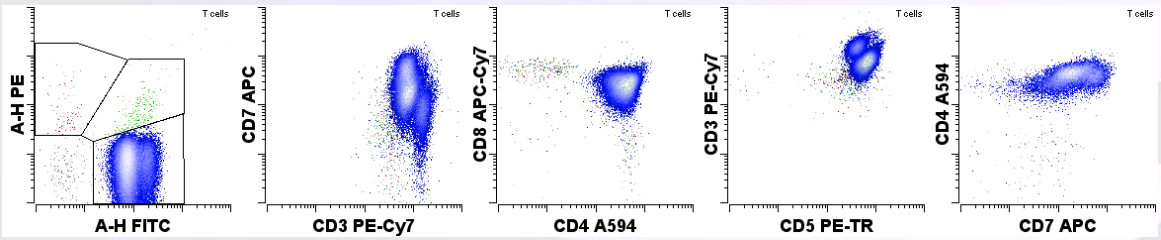
T cell clonality

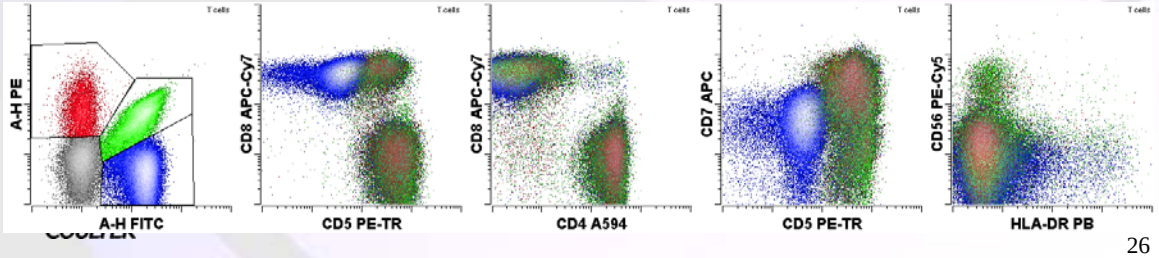




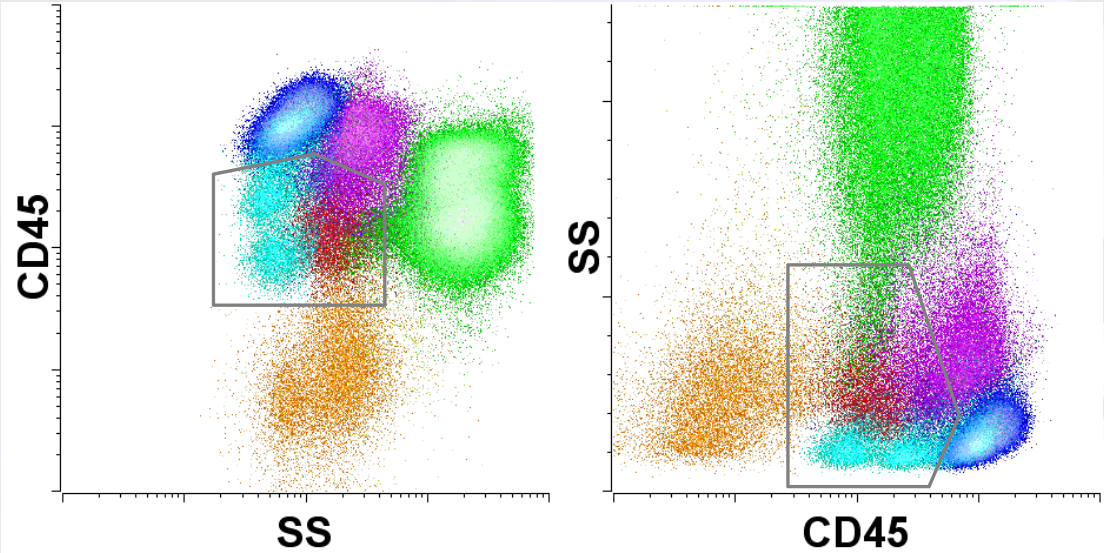
T cell clonality

- All TCR-beta reagents in single tube
  - Efficient, good for small specimens

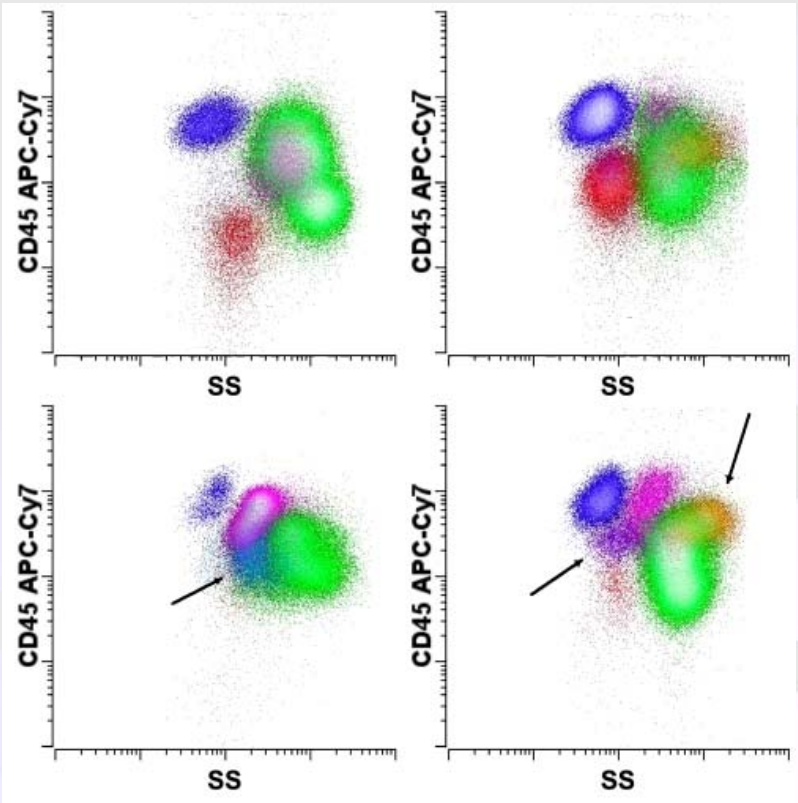




Cell Type Identification

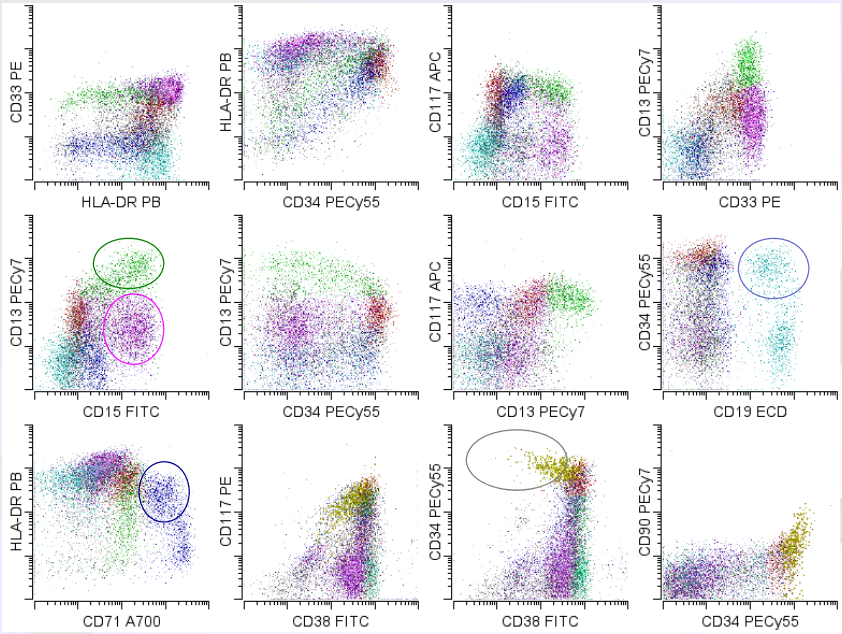


Borowitz et al (1993) AJCP 100:534-40.
Steltzer et al (1993) Ann NY Acad Sci 667:265-280



Wood (2007) Clinics in Lab Medicine 27:551-575

Normal Blast Maturation



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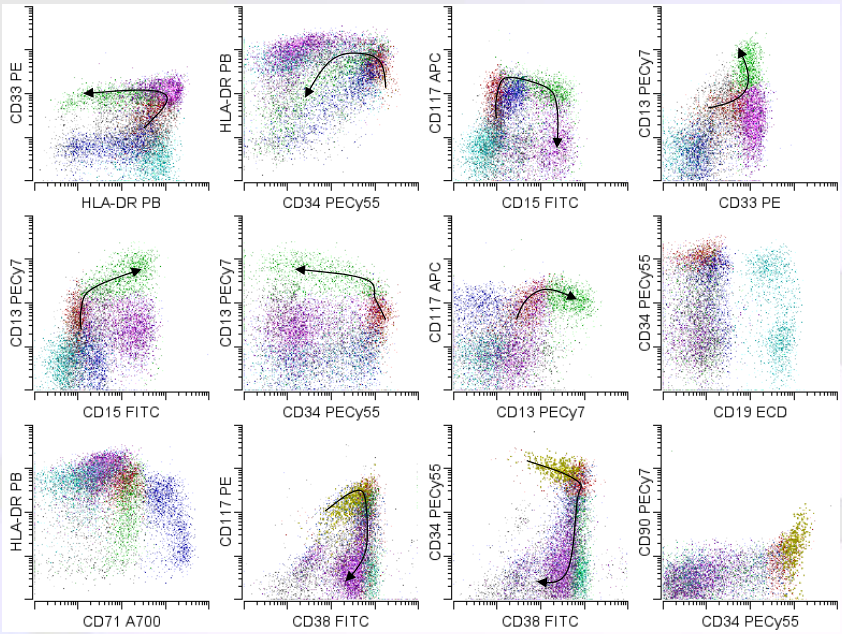
Wood (2004) Methods Cell Biology 75:559-576

Normal Granulocyte Maturation



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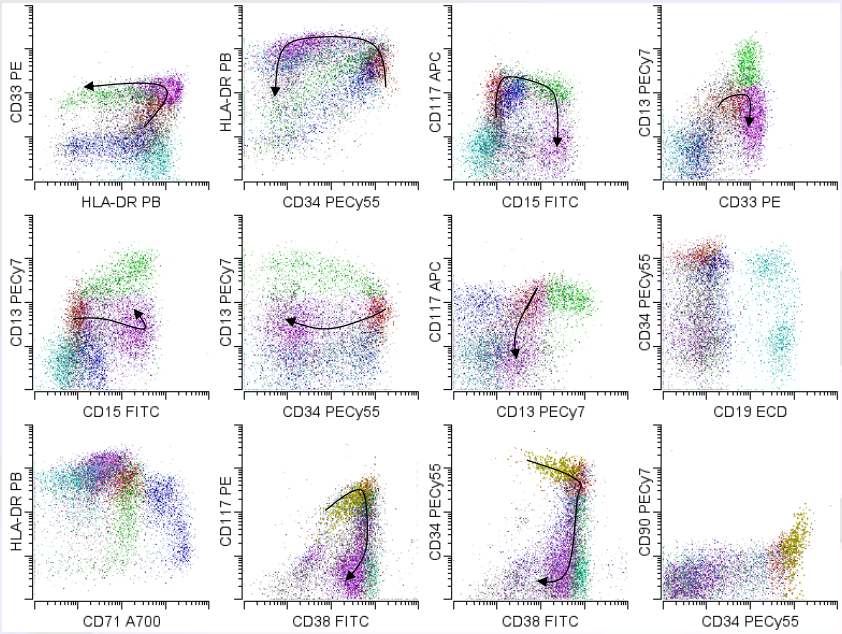
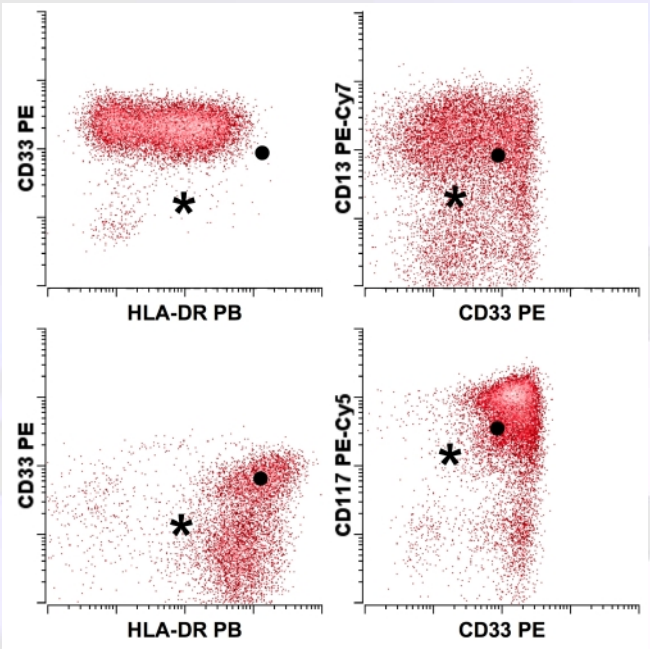
Normal Monocyte Maturation

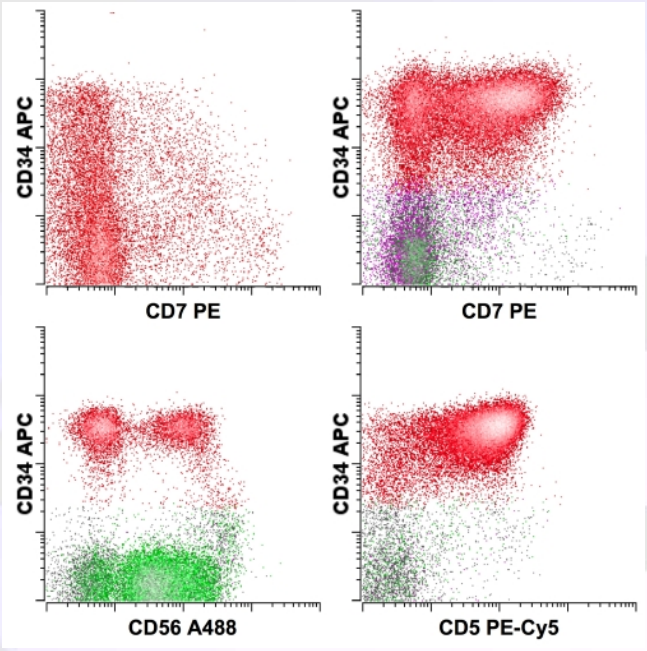


Blasts - Abnormal Antigen Intensity



Wood (2007) Clinics in Lab Medicine 27:551-575

Blasts - Aberrant Lymphoid Antigens



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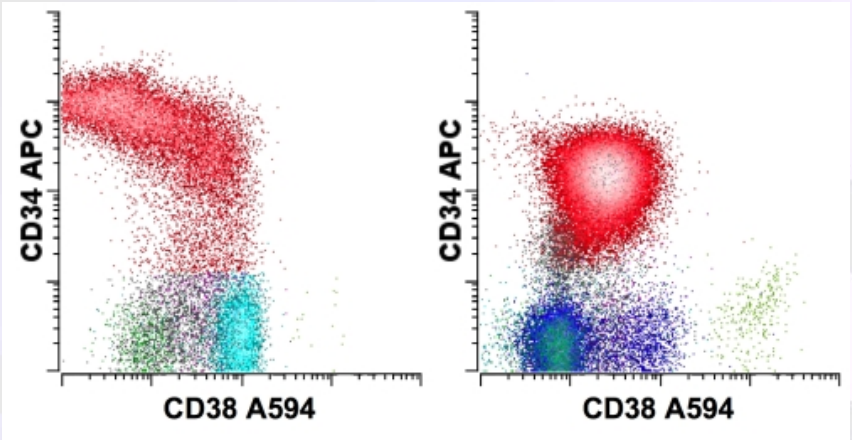
Wood (2007) Clinics in Lab Medicine 27:551-575

Blasts - Aberrant Maturation



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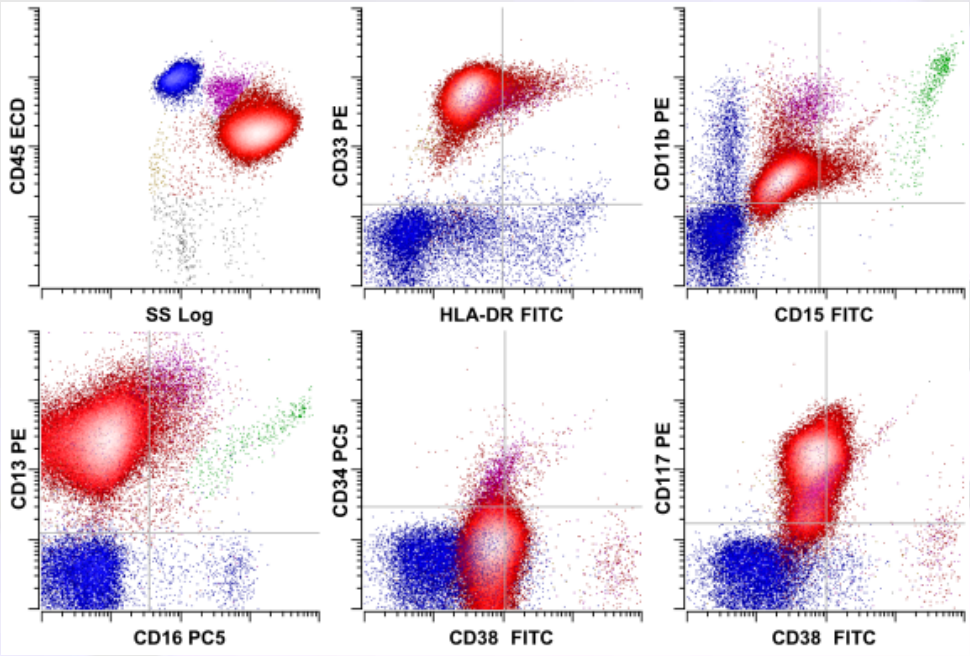
Wood (2007) Clinics in Lab Medicine 27:551-575

Acute Promyelocytic Leukemia



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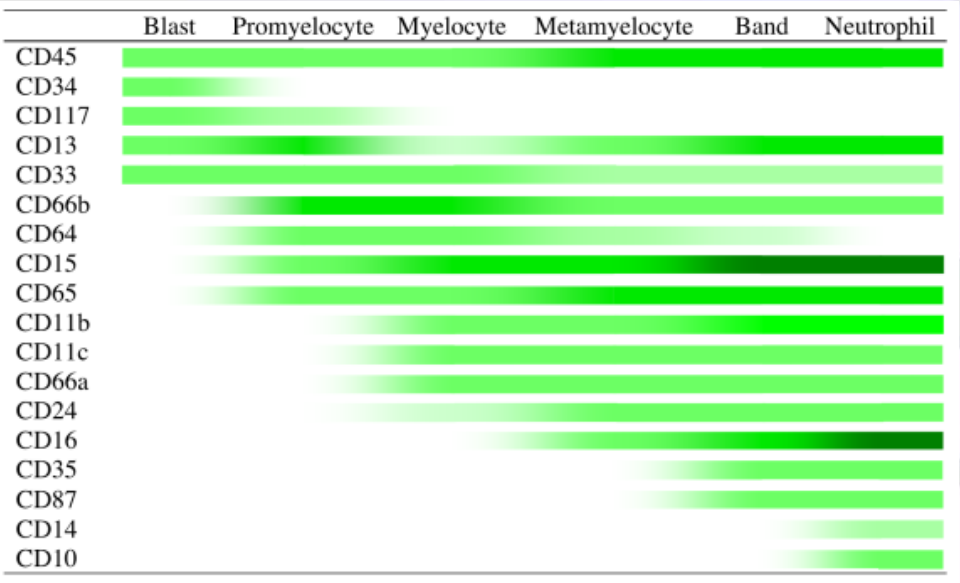
Wood and Borowitz (2006) Henry's Laboratory Medicine

Normal Granulocytic Maturation



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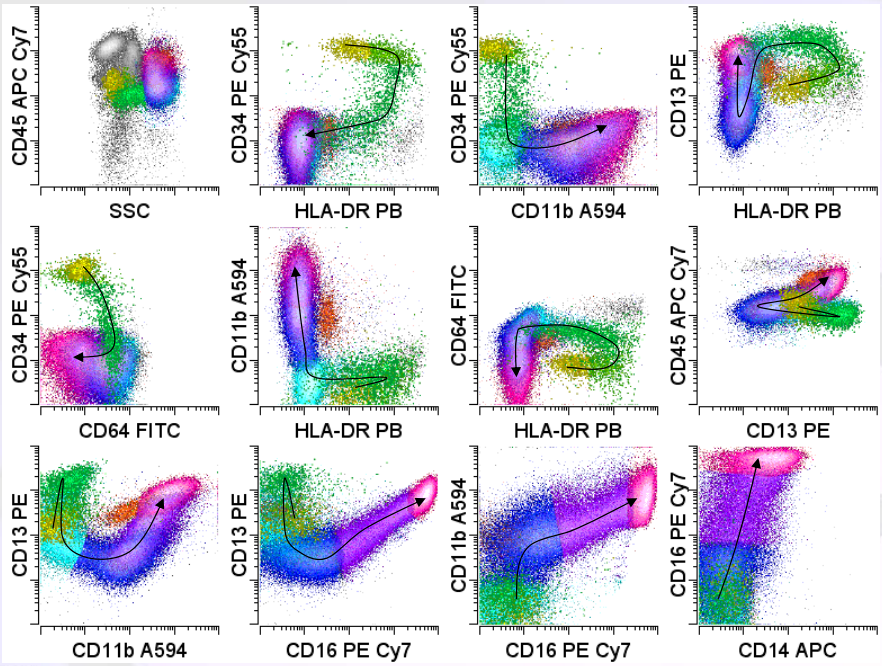
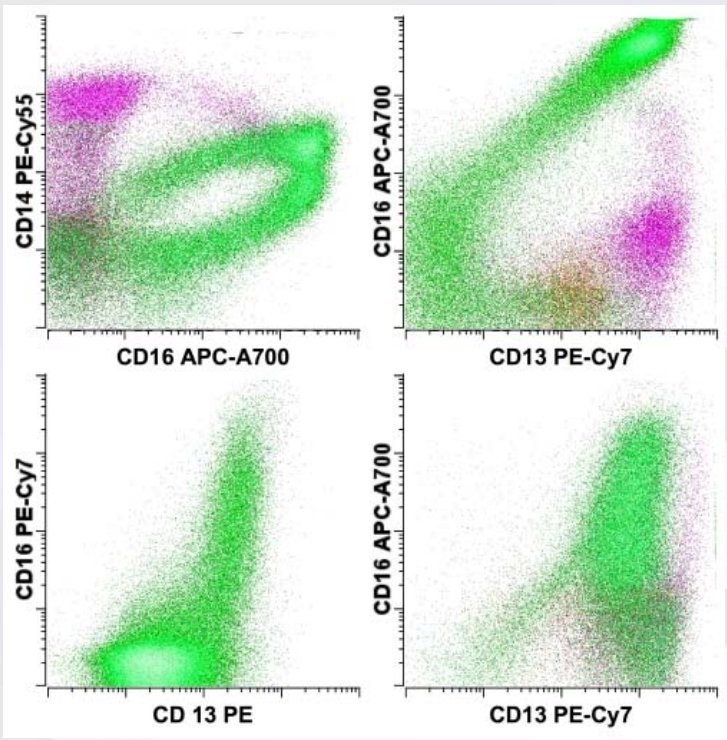
Wood and Borowitz (2006) Henry's Laboratory Methods

Normal Granulocytic Maturation

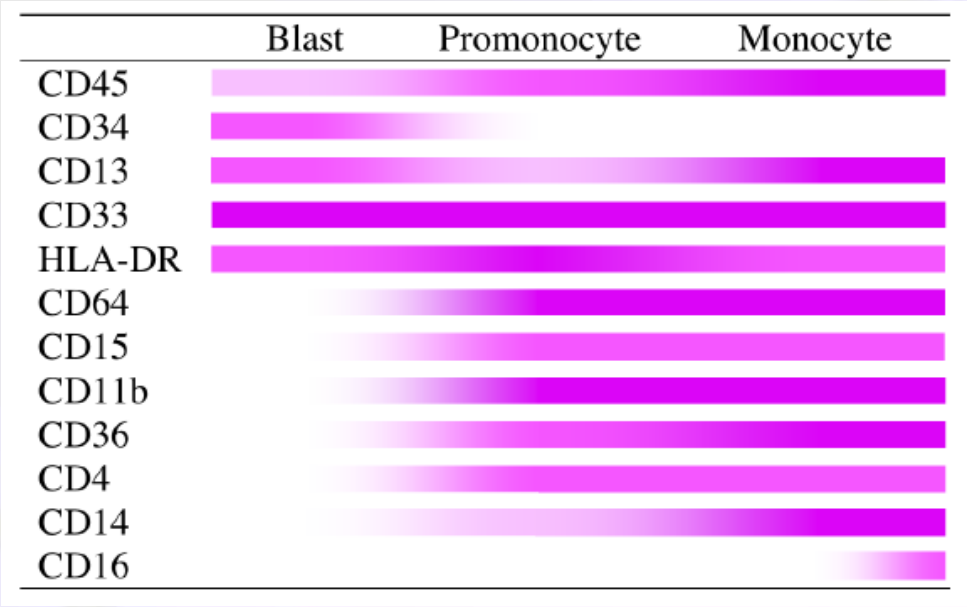


Wood (2004) Methods Cell Biology 75:559-576



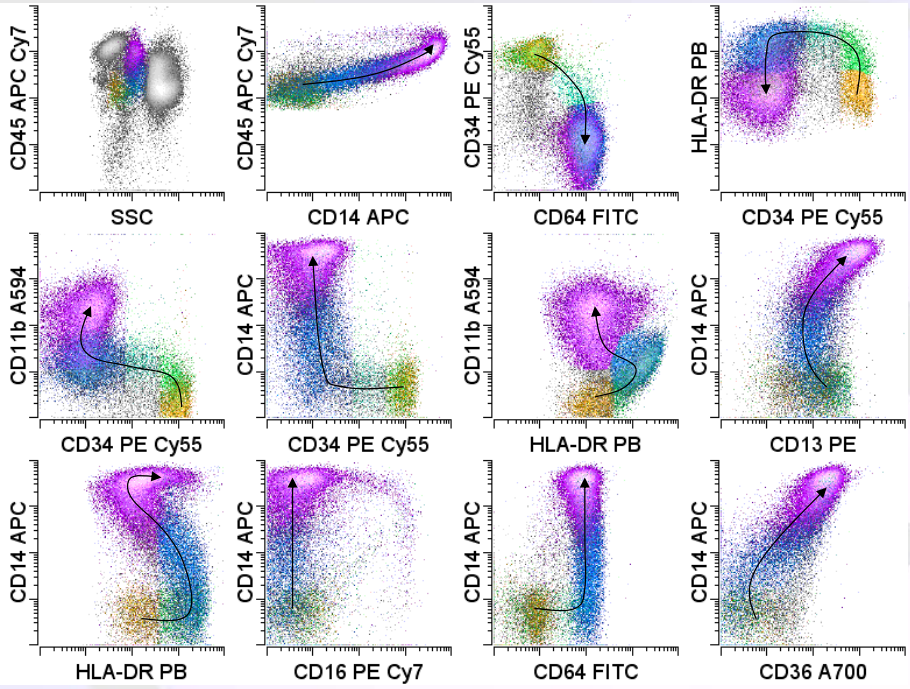
Wood (2007) Clinics in Lab Medicine 27:551-575

Normal Monocytic Maturation



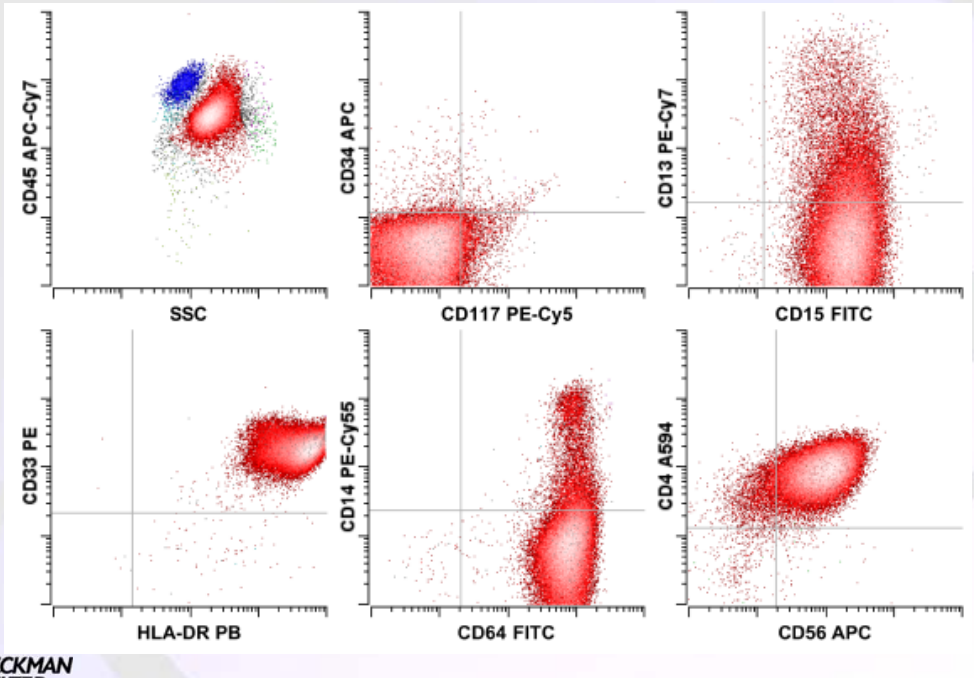
Wood and Borowitz (2006) Henry's Laboratory Methods

Normal Monocytic Maturation



Wood (2004) Methods Cell Biology 75:559-576

Acute Monocytic Leukemia



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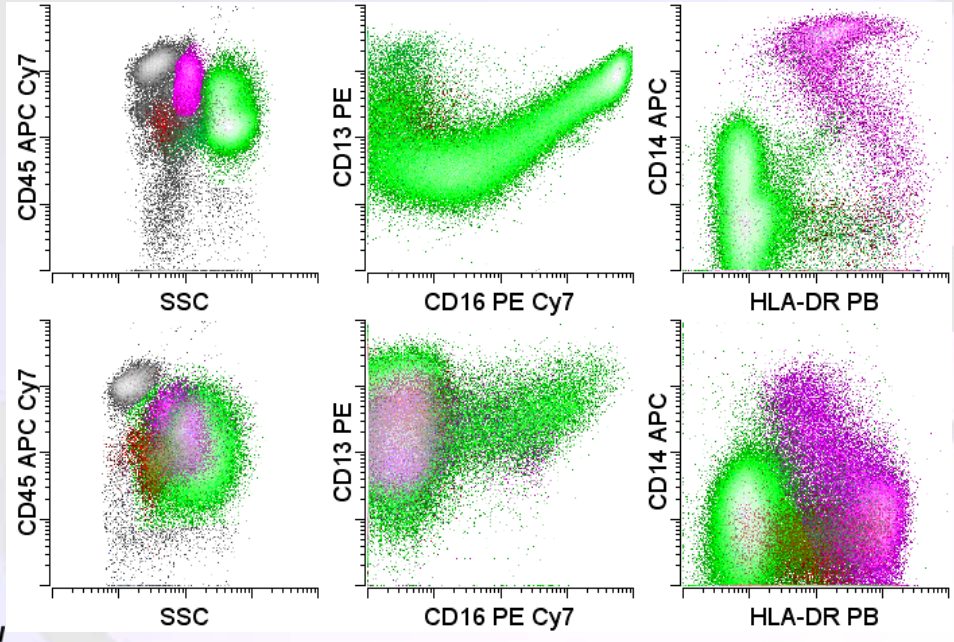
Abnormal Myeloid Maturation

Normal

MDS



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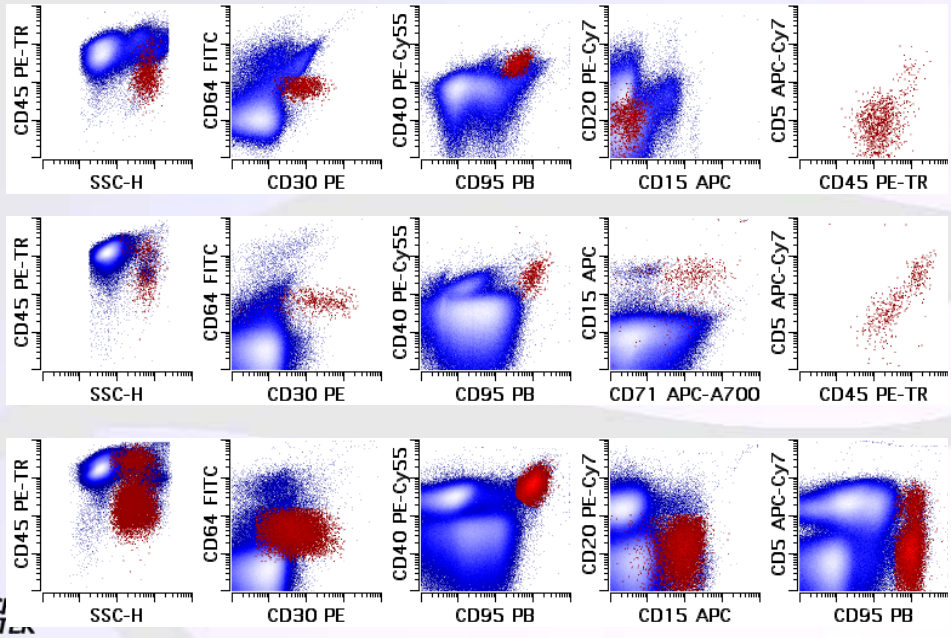
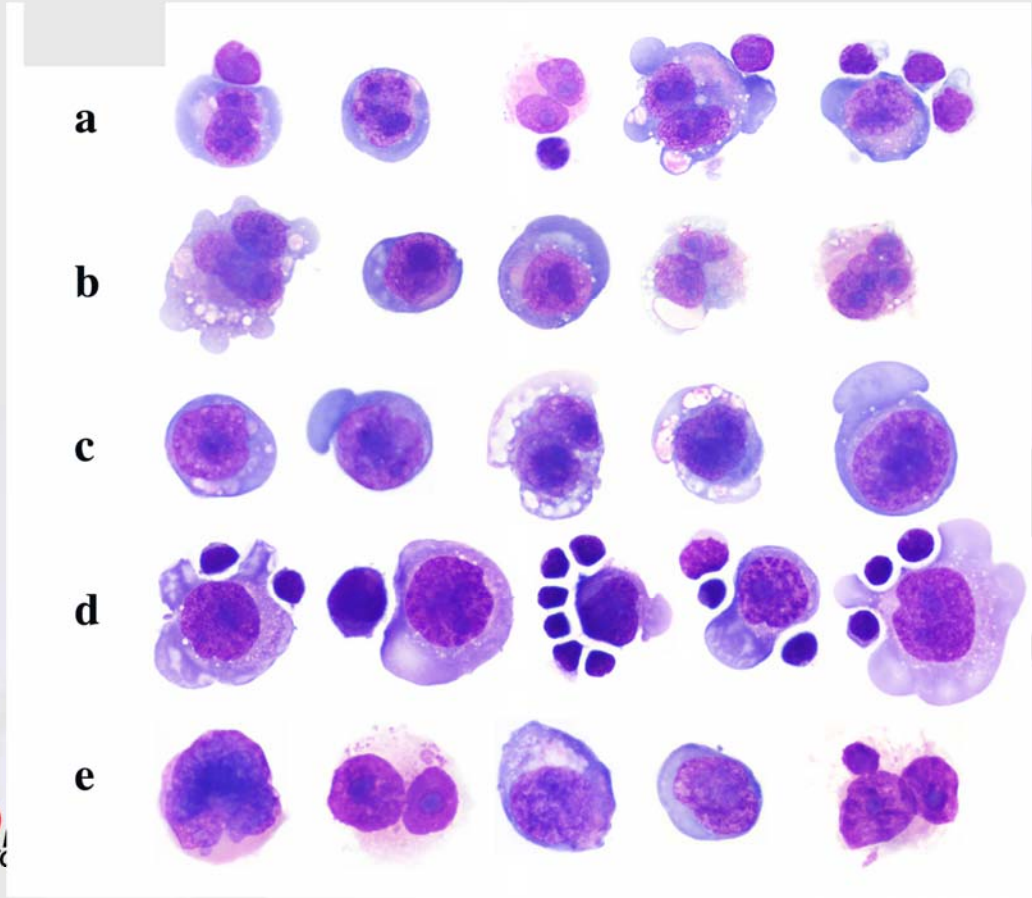
Classical Hodgkin Lymphoma





Deficiencies

- Requires an expert
  - Understand normal maturation
- Increasing number of parameters = increasing complexity
  - Simplification by comprehensive population identification
- "Pattern recognition" subjective
  - Inherits poor objectivity of morphology

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Data Analysis

Need improved software to analyze high-level multiparametric data

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Conclusion

- High-level multicolor flow cytometry can be successfully performed in a clinical setting
- Offers advantages in efficiency, informational content and standardization
- Standardization and optimization of instrument performance, reagents and analysis strategy is critical

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